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OBSERVATIONS ON HOST-PARASITE RELATIONS BETWEEN LABORATORY MICE AND *NEMATOSPIROIDES DUBIUS* BAYLIS*

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The writer undertook a series of infection experiments using highly inbred strains of laboratory mice and *Nematospiroides dubius* Baylis. This nematode (Family HELIGMOSOMIDAE) is a normal parasite of wild house mice. The experiments were conducted through 1940 and the spring of 1941 at Davis, California.

MATERIALS AND METHODS

Three strains of mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. These strains were all highly inbred by brother-sister matings so that a high degree of homozygosity could be expected in each. There were no visible size differences among the animals of each strain. The strains consisted of the following:

(1) C–57 stock, inbred 20 generations; black in color; of genetic formula *aa;* characterized by mammary carcinoma in about 1% of the breeding females, none in virgins; various types of internal tumors, mostly sarcomas, 10–20% in both sexes, high fertility;

(2) A–W stock, white-bellied agouti, inbred 20 generations, genetic formula A^wA^w; medium fertility;

(3) L stock, inbred 40 generations, non-agouti brown leaden, genetic formula *aabbd2d2;* derived by mutation from C–57 br (which was derived by mutation from the C–57 strain) of medium fertility.

According to Babcock and Clausen (1927) matings of brother and full sister represent the most effective method of promoting homozygosity in animals. Their Fig. 195 (adapted from Wright) shows about 98 per cent homozygosity in all characters to be attained after 16 generations of brother-sister matings. On this premise the strains used in the present experiments should be 98 per cent or more homozygous in all genetic characters, since all derive from brother-sister matings through at least 20 generations. Mice have 36 chromosomes. These mice, after their arrival on August 12, 1939, were mated at random within each strain in order to multiply their numbers, and this practice was followed in all subsequent generations.

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The mice were housed in screen-top galvanized pans provided with sawdust and shavings. Water was offered in suspended bottles.

The diet consisted of dog chow in large checkers (presumed to be an adequate diet), and supplemented by one feeding of carrots and one of alfalfa alternating at weekly intervals.

Infected animals were housed and fed as was the normal colony, except that the litter was changed twice weekly to guard against completion of the parasite life cycle. Uninfected control mice were caged with test animals throughout all experiments. The cages were cleaned with a metal scraper and then rinsed in 2% lysol solution as a further safeguard against survival of infective larvae. It was previously determined that infective larvae are killed by exposure to 2% lysol for one minute.

The original stock of *Nematospiroides dubius* used in these experiments was taken from the small intestines of wild house mice (*Mus musculus musculus* Linnaeus) collected November 25, 1939, in a rice field on the Conway Ranch near Davis, California.

The life cycle of *N. dubius* will be described in detail in another paper. The following summary outlines the features essential to understanding the experiments to be described.

Eggs of *Nematospiroides dubius* are carried out in the feces of the host, hatch, and pass through the free-living larval stages in moist fecal material. The sheathed infective larvae migrate to the surface of the fecal mass by the 4th to 6th day after fecal deposition. Such larvae upon being ingested by a host mouse, molt while in the lumen of the intestine, then migrate through the mucosa, and come to lie in loose cysts in the circular and longitudinal muscle layers of the intestinal wall. There the last molt occurs, the young adult worms returning to the lumen of the intestine, where mating and oviposition take place. The entire cycle, from egg to egg, is completed in 13 to 15 days in the A–W strain of mice and fecal counts may be positive from 7 to 8 months following infection.

Larvae were washed from fecal cultures on the fifth or sixth days from the time of culturing, rinsed twice, and left in clean tap water.

Infection was accomplished by administering a counted number of infective larvae with a small pipette to mice deprived of drinking water for 24 hours. The pipette was next filled with clean tap water, also given to the mouse, then the pipette was rinsed and the rinse water checked beneath a binocular dissecting microscope to be certain that all of the larvae had been administered. Restraint of the mice while being infected was unnecessary; being thirsty, they licked avidly at the end of the pipette.

The larvae used in experimental infections were all from cultures of 6 to 15 days of age. These age limits were used to avoid any effects of age in different cultures of larvae. The same procedure was followed by Africa (1931) in his experiments with *Nippostrongylus muris*.

To obtain autopsy data, the mice were sacrificed, the viscera removed, and the small and large intestines were slit open. Any worms present were removed under a binocular dissecting microscope. The live worms were kept in 0.7% NaCl overnight, and counted the next day under the dissecting microscope, then preserved in 10% formalin.

In the experiments on acquired resistance to superinfection the worm measure-

ments were made by outlining the length of the worm under a camera lucida and measuring it with a calibrated wheel.

MORTALITY EXPERIMENTS

Fecal examinations were conducted on 32-day-old mice of the L, A–W, and C–57 stocks after infection with 100 larvae each of *Nematospiroides dubius*. The egg count data, however, are inconclusive and the writer feels that larger numbers of animals should be egg-counted before entirely reliable data can be obtained. In his experiments only 6–10 test animals plus 1 control could be egg-counted in each strain.

In the A–W strain of mice mortality to the dosage of 100 larvae was noted during the egg-count period while none occurred in other strains. Consequently further infection experiments were set up in which 32-day-old mice were infected with 100 larvae each of *N. dubius*. In addition one group of 26 A–W mice 32 days old were given 500 larvae each. Controls were run to equal half the number of test animals within each group and at the end of each experiment were either autopsied or egg-

TABLE 1.—*Mortality percentages*

Strain of mice	No. of mice	Larvae given	% mortality
L	19	100	0
C–57	25	100	0
A–W	50	100	26
A–W	26	500	100
Control mice same age	Approx. 50% of each group	0	0

counted 3 times to be sure that infection had not occurred in them. The results are given in Table 1. The 50 A–W mice given 100 larvae each showed a 26% mortality with the deaths occurring from 15–35 days after infection and the 26 A–W mice given 500 larvae each showed 100% mortality with deaths 8–23 days after infection. No further deaths were observed in either group up to the 50th day after infection or in the other strains or controls for the entire period.

Using mortality as a criterion the author concludes therefore that the A–W strain is more susceptible to the infection of 100 larvae than are the L and C–57 strains.

Symptomatology.—A–W mice were the only ones showing symptoms of infection. These began to weaken 1–10 days before death. Progressive emaciation, sensitivity to cold and roughness of coat were always present. The mice showed a reddish-brown to watery diarrhea, the feces often forming a large anal plug at death. Appetite was retained in some instances up to within a few minutes of death, which occurred on the 16th to 35th day following infection.

Post-mortem findings.—Lungs were normal. There was a slight serous to serosanguinous exudate in the body cavity; liver and kidneys were light colored, sometimes bleached or pallid; the spleen was often enlarged and light pink; the small intestine was enlarged, especially in its anterior 2 inches, fragile, light in color, and transluscent. Appearance of the abdominal cavity was indicative of a severe anemia.

The stomach, small and large intestines were filled with food. The intestinal lumen often contained blood clots, especially in animals dying early in the infection period; the mucosa was severely inflamed or with heavy petechial hemorrhage, particularly about the heaviest concentrations of worms; the latter were usually grouped anteriorly in duodenum but in more chronic cases were scattered throughout the

tract, and in few instances, were observed passing out through the anus in large numbers. There was perforation of the anterior duodenum in severe cases with death from peritonitis. The large intestine and cecum were filled with feces, usually of a dark chocolate color and giving positive benzidine test for blood. The anus was often blocked with a firm, dried fecal plug.

STRAIN COMPARISON BY INTERVAL AUTOPSIES

Infection Experiments in 32-day-old Mice

A–W and C–57 mice were infected at the age of 32 days with 500 larvae each. Autopsies were conducted 7, 11, 18, 25, and 32 days respectively following infection. The average numbers of worms recovered from each strain of mice at the various autopsy intervals are given in Table 2. None of the A–W mice survived up to the 18th day following infection, whereas the C–57 mice showed no mortality up to the 32nd day following infection, when the last of the latter were sacrificed. There is no significant difference between the two strains of mice as to the number of worms recovered. In Table 3 the worm populations of the animals in Table 2 for the 7th

TABLE 2.—*Total worm recovery averages with probable error of the mean; dosage was 500 larvae per mouse; mice 32 days old*

Days after infection	7	11	18	25	32
A–W strain, 5 mice per period	478 ± 2.5	478 ± 3.1	*	*	*
C–57 strain, 5 mice per period	444 ± 2.8	456 ± 2.1	451 ± 3.7	459 ± 3.6	406 ± 26.8

* 30 more A–W mice died of the infection within 18 days after infection. The worm population in these animals was not ascertained.

TABLE 3.—*Average worm distribution from Table 2*

Days after infection	7		11	
Worm recovery percentages	From cysts	From lumen	From cysts	From lumen
A–W strain	87 ± 0.7	13 ± 0.7	*	100
C–57 strain	74 ± 0.5	26 ± 0.5	*	100

* Less than 0.1.

and 11th days have been broken down to show the distribution of their occurrence within the host. Since a larger percentage of the worms are able to pass through the encysted stage on the intestinal wall by the 7th day in the C–57 as compared to the A–W strain it is apparent that there is some factor or factors operating in one of the other of these strains which either speeds up the encysted phase of the life cycle in the C–57 strain or slows it down in the A–W mice. This factor, however, is not apparent on the 11th day following infection.

Autopsy findings: Seven days following infection of A–W and C–57 mice.—Cysts containing 1–12 small reddish worms were found in the muscular coat of the small intestine just under the serosa; these were most numerous in the first 2 inches of intestine but were also scattered posteriorly, in a few instances within ½ inch of the iliocecal valve. No other pathological changes evident. Some worms were found migrating back into the intestinal lumen; empty cysts were seen filled with blood or as white discs against the pink intestinal wall.

Eleven days following infection of A–W and C–57 mice.—The abdominal cavity was almost normal. The intestinal wall in some cases was soft and glandular

in appearance with small blood-filled cysts; the duodenal wall often showed hemorrhagic spots at points of entrance of worms into the lumen. The mucosa occasionally had a "frosty" appearance, which was more pronounced later in the infection period. "Frostiness" was due to the color of the villi and was most evident in areas of worm concentration. Some animals already exhibited splenomegaly with some paling of the normal red color of spleen and also of the liver and kidneys. Symptoms were more pronounced in A–W strain.

Eighteen to 32 days following infection of C–57 mice.—The animals autopsied on the 18th day showed splenomegaly, with the spleen pale and rather cloudy in appearance; liver and kidneys were normal. The duodenum was enlarged, especially anteriorly, and glandular from gross exterior view; internally the mucosa was petechiated, frosty in appearance at the site of worm concentrations with some erosion of villi in duodenum; duodenal contents were normal except for excessive amount of liquid. On 25th day the spleen was normal or but slightly enlarged, but often dark with some cloudiness which imparted a bluish cast. Duodenum was similar in appearance to that on the 18th day.

On 32nd day the findings were the same as on the 25th day.

A–W mice dying after infection.—Those dying from infections of 500 larvae given at 32 days of age usually had symptoms and post-mortem findings similar to those with a dosage of 100 larvae. Death was sudden after onset of symptoms or sometimes without symptoms. Mortality started and ended earlier, 8–23 days following infection, and the pathological changes in the intestine were more pronounced than in mice given 100 larvae.

There was little or no evidence of diarrhea; intestinal trauma and hemorrhage was extensive; cysts on intestinal wall had few or no worms; empty cysts appeared as white to greyish discs, sometimes filled with blood and bright red; the spleen was not always enlarged, especially in those dying on 9th–12th days; in those dying later the spleen was sometimes twice normal size and light pink in color, and usually cloudy in appearance.

Infection Experiments in 5-month-old Mice

A–W and C–57 mice 5 months of age were infected with 500 larvae each of *Nematospiroides dubius*. Autopsies were conducted at 7, 11, 18, 25, and 32 days respectively following infection, using the same procedure as in the earlier experiments with 32-day old A–W and C–57 mice.

According to the experimental procedure here followed, it should be possible to compare 32-day and 5-month mice of both A–W and C–57 strains for a measure of age resistance. Two criteria are available as measures of resistance: (1) total worm recovery, and (2) rate of passage through the cyst phase in the worm life cycle. As a criterion of the latter the per cent of the total population present in the lumen of the intestine and the per cent still retained in cysts on the intestinal wall were determined for the 7th and 11th days respectively after infection. The latter criterion was chosen because of its ease of determination in the life history of this nematode.

Table 4 shows the average number of worms recovered from the two strains of mice at the several autopsy periods following infection. There is a significant difference between the number of worms recovered in the two strains, more worms having been recovered from the C–57 strain.

In Table 5 the worm populations of the animals in Table 4 for the 7th and 11th days have been broken down to show the distribution of their occurrence within the host. Five per cent of the worm population has reached the lumen by the 7th day in the A–W strain as compared to 64% in the C–57 strain and 94% on the 11th day as compared to 100% in the C–57 strain. The worms in the A–W strain are proceeding through their encysted stage more slowly than are those in the C–57 strain.

TABLE 4.—*Total worm recovery averages with probable error of mean; dosage was 500 larvae per mouse; mice 5 months old, 5 mice per period*

Days after infection	7	11	18	25	32
A–W strain, 2 died out of 26	411 ± 3.6	409 ± 6.6	416 ± 9.1	427 ± 5.8	356 ± 9.4
C–57 strain, no deaths	459 ± 2.1	469 ± 4.8	*	*	*

* Autopsies were not conducted upon the C–57 strain for the last 3 periods.

TABLE 5.—*Average worm distribution from Table 4*

Days after infection	7		11	
Worm recovery percentages	From cysts	From lumen	From cysts	From lumen
A–W strain	95 ± 0.5	5 ± 0.5	6 ± 1.7	94 ± 1.7
C–57 strain	36 ± 0.6	64 ± 0.6	*	100

* Less than 0.1.

ACQUIRED RESISTANCE TO SINGLE SUPERINFECTION

To test the effect of a residual single initial infection upon a subsequent infection, 27 A–W mice were used in 3 groups (Table 6). Group A (6 mice) 32 days of age, was infected with 50 larvae per animal and autopsied at the age of 79 days. Group B (6 mice) 64 days of age, was infected with 100 larvae per mouse and autopsied at 79 days of age. Group C (15 mice) 32 days of age, was infected with 50 larvae each and again at 64 days of age with 100 larvae each, and the mice were autopsied at 79 days of age.

Groups A and B served as controls for the test animals of Group C, since Group A received only the initial infection, Group B only the second infection, and Group C had been given both the initial and subsequent infections. Therefore, if one initial infection of 50 larvae has any effect upon the number of worms recovered from the second infection, the average recovery from Group C should be less than that from Group A plus Group B.

The average worm recovery for each group in the A–W strain is given in Table 6. That of Group A was 41 worms, of Group B 96 worms (A and B combined, 41 + 96 = 137), and of Group C was 134 worms. Obviously, the two values, 134 and 137, are not significantly different.

The foregoing experiment was duplicated using C–57 instead of A–W mice. The results are given in Table 7. The average recovery was: Group A, 47 worms; Group B, 96 worms (A and B combined, 47 + 96 = 143), and Group C, 138 worms. Again there was no significant difference between test and control animals.

Length measurements were made of worms from each group of mice in the two experiments just described. Only males were measured because the female worms are coiled too tightly to make such measurements feasible. Length, as used here,

is the distance from the anterior end of the body to the anterior margin of the bursa. In the A–W strain, ten male worms were measured from each mouse of Groups A and B and ten worms from each of the first 6 mice in Group C. The lengths of the worms from Groups A and B were averaged to avoid possible effects of age differences in the worms. The worms of Groups A and B averaged 5.18 mm, and of Group C, 4.84 mm. A chi square comparison of these figures gives a value of 0.0111, not significant.

Such measurements were repeated for worms from the C–57 mice. Those of Groups A and B, averaged together, were 5.14 mm, of Group C, 5.10 mm. Again the difference has no significance ($X^2 = 0.0002$). It can be concluded, therefore, from the number and length of worms recovered, that no resistance was acquired from one residual primary infection of 50 worms in either strain of mice.

TABLE 6.—*Lack of acquired resistance to single superinfection—A–W strain*

	Dosage at 32 days of age	Dosage at 64 days of age	No. of worms recovered at 79 days of age
Group A, 6 mice	50	0	41 ± 1.5
Group B, 6 mice	0	100	96 ± 0.5
Group C, 15 mice	50	100	134 ± 1.2

A + B = 137

TABLE 7.—*Lack of acquired resistance to single superinfection—C–57 strain*

	Dosage at 32 days of age	Dosage at 64 days of age	No. of worms recovered at 79 days of age
Group A, 5 mice	50	0	47 ± 0.4
Group B, 5 mice	0	100	96 ± 1.1
Group C, 10 mice	50	100	138 ± 0.8

A + B = 143

At autopsy, Group A of each strain showed little pathological change, the only abnormality being a slightly "frosty" appearance of villi in areas of worm concentration. Group B of each strain showed some splenic enlargement, lightly frosted duodenal mucosa with petechiation, slight erosion of villi. Group C showed dull colored and slightly enlarged spleen; the duodenum was rather rough and glandular in appearance from exterior view; empty cysts on outside of the intestine were more distinct than in Group B, in some cases containing white to yellowish, caseous material.

DISCUSSION

It is clear from the foregoing experiments that the A–W strain of mice was very susceptible to a dosage of 100 larvae of *Nematospiroides dubius*. It exhibited a 26 per cent mortality when infected at that dosage while the other strains showed no mortality (Table 1). On the other hand, with a dosage of 500 larvae the mortality was 100 per cent in the A–W strain and started and ended earlier in the infection period.

In addition to the difference in mortality between the C–57 and A–W strains the worms passed through the encysted phase faster in C–57 mice 32 days old than in A–W mice the same age. In 5-month-old mice of the two strains this differential in rate of cyst passage was more pronounced.

When old and young mice were compared in the interval autopsy experiments the total recovery in 5-month-old A–W mice was somewhat less than in 32-day-old

mice of the same strain (Tables 2 and 3). In the C–57 strain there was no significant difference in total recovery between the two age groups.

A significantly smaller percentage of the total worm population was able to reach the lumen of the intestine by the 7th day in the 5-month-old A–W mice as compared to the 32-day-old mice of the same strain (Tables 3 and 5). With the C–57 strain the reverse was true, a larger percentage reaching the lumen on the 7th day in old as compared with young mice.

Unfortunately no experiments were carried out based upon a standard worm dosage per gram of body weight so that some of the differences between the two age groups may be due to differences in size or weight. However, not all the differences are ascribed to this factor since the rate of completion of the encysted stage was different in the two strains, having been slowed down in the A–W mice and speeded up in the C–57 strain with increasing age.

The A–W strain is susceptible to the infection on the basis of mortality as compared to the C–57 strain. However, since the parasite passes through its encysted stage slowly in the A–W strain as compared to the C–57 strain, the A–W strain might be considered resistant on this criterion. It is possible that the A–W strain is susceptible to the effects of the parasite but shows an actual resistance to the parasite itself. With the C–57 strain the reverse could be true, i.e., resistant to the effects but susceptible to the parasite. Thus it could be a more favorable host than the A–W strain. The fact that the C–57 strain allows the cyst phase of the life cycle to be completed more quickly in 5-month-old animals as compared to 32-day-old mice seems to point to its becoming a more favorable host with increasing age. This latter phenomenon then, may possibly be an inverse age resistance.

The differences noted between the strains of mice studied here seem most clearly explainable on a genetic basis since the different strains used were highly inbred and hence highly homozygous.

Among other workers, Graham and Porter (1934), Ackert (1935), Cameron (1935), Sheldon (1937), and Stewart, Miller, and Douglas (1937) have reported what may be genetic differences in animal hosts in relation to their parasites.

Little can be said of acquired resistance from the experiments reported here. Eliciting or detecting an acquired response might necessitate a larger initial dosage, several dosages, or other criteria of resistance.

SUMMARY

By infecting 3 highly inbred strains of laboratory mice with a standard dosage of *Nematospiroides dubius* larvae the writer has shown that the A–W strain is highly susceptible to the infection on the basis of mortality.

The C–57 strain is resistant on this basis but is a favorable host using as a criterion the rate at which the parasite passes through its encysted stage in the intestine of the mouse.

The C–57 strain on the latter criterion is a more favorable host at 5 months than at 32 days of age while the reverse is true of the A–W strain.

On the basis of total worm recovery the A–W strain is slightly more resistant at 5 months than at 32 days of age although part of the age differences in these strains may be due to increase in weight of the mice.

The strain differences demonstrated here appear to be most easily explainable on a genetic basis.

No conclusions are drawn regarding acquired resistance except that it was not observed under the conditions of this experiment.

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A CONTRIBUTION TO THE KNOWLEDGE OF THE RODENT WARBLE FLIES (CUTEREBRIDAE)

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The rodent warble flies are myiasis-producing Diptera restricted to North and South America, the larvae of which inhabit the subcutaneous tissues mainly of rodents, lagomorphs, and small domestic animals. The adult flies, which are rarely observed in nature, have reduced mouthparts and do not feed.

References to the habits or biology of these flies are scarce, even though larvae are frequently encountered by veterinarians and others, especially in relation to infections of cats and dogs.

The author has been informed by veterinarians from various parts of the United States that these larvae are commonly found in cats and dogs. Personal reports from midwestern states, particularly from Ohio, indicate that it is common practice for owners to remove larvae from beneath the skin of pets every summer, and the Small Animal Clinic, New York State Veterinary College, Ithaca, N. Y., is frequently called upon to remove such larvae. These cases are sufficiently common that Cornell veterinarians approximate the infection period as early July to late September, with a peak from mid- to late August. At the New York State Fair at Syracuse in 1939, many people observing the exhibit of parasites of domestic animals, were overheard saying that their cats and dogs often get such infection during the summer.

Many workers have conjectured that infection of cats and dogs is solely accidental and occurs only when these animals eat infected rodents and lagomorphs. However, nursing kittens are often found infected. Dr. H. H. Schwardt (Cornell University), and others, have found their house cats, which never roam through the fields, infected with *Cuterebra horripilum* Clark, a species usually infecting cottontail

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[1] The author wishes to thank Mr. William M. Rogoff who has given many helpful suggestions during the preparation of this paper.

rabbits. Such findings, as well as many *Cuterebra* infections of cats and dogs seen by the author, indicate that these animals may commonly serve as hosts of *Cuterebra* species.

A few cases of *Cuterebra* infection have been recorded from opossums, hogs, and asses, and one case from man. The author has one mature larva of *Cuterebra horripilum* removed from the trachea of a pig at the New York State Veterinary College in August, 1942. These larger animals, however, are not frequently found infected with cuterebrae, and the unusual points of localization of the larvae in these hosts indicate that such infections are accidental.

Besides such circumstantial evidence, much experimental field and laboratory work must be done before we know accurately the mode of infection of these parasites, their path, if any, through the body, and the effect upon the host.

OBSERVATIONS

The following observations of *Cuterebra* infections were made by the author during 1940 and 1941 at Ames, Iowa. Extensive trapping was carried on of the north-

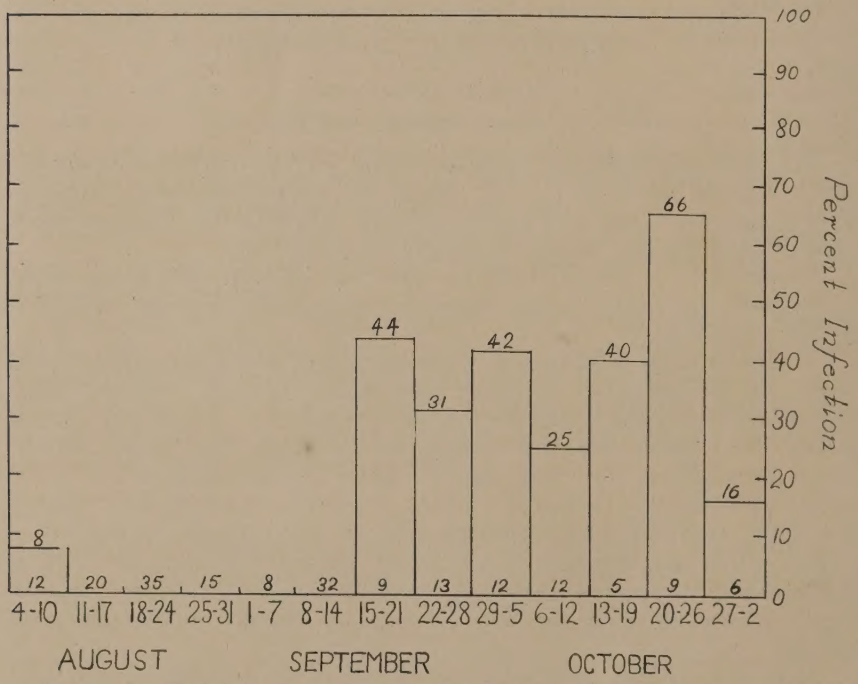

FIG. 1. Seasonal infection of northern white-footed mouse with *Cuterebra peromysci* during 1940. Numbers at bases of columns represent the numbers of mice caught for the corresponding week periods.

ern white-footed mouse (*Peromyscus leucopus noveboracensis*) which was found to act as the host of the larvae of *Cuterebra peromysci* Dalmat (1942). Traps were set daily from early summer to mid-winter during 1940. The infection period extended from September 15 to November 2, during which time 25 of the 68 mice caught (38

per cent) were parasitized. Excluding the one parasitized mouse trapped August 4, infection was not encountered at any other time during the trapping period (Fig. 1). It will be noted that more mice were caught during the period in which no infection was found.

In 1941, 54 per cent of the mice trapped in the same area were found to harbor *Cuterebra* larvae. The larvae were located predominantly in the general inguinal region, each larva with its posterior spiracular plates exposed through a hole in the host's skin. Several mice harbored between three and five of them and one mouse bore seven in different stages of development.[2] (One of these larvae was a first instar.) Young mice were seldom found parasitized. Almost all infected animals were awkward, many of them attempting to remove the bulging larvae with their claws and teeth. When a male mouse harbored more than one parasite, total emasculation usually resulted. About 20 per cent of the infected mice were found dead in their cages at about the time the larvae started to emerge.

Although infection by these parasites is often high, it is local in distribution. Almost all the infected mice were caught by the author in an area about four acres in size, while similar types of areas about 500 yards away with numerous mice yielded no infection. This has also been the experience of others who encounter *Cuterebra* in field animals but is not surprising as most species of warble flies, although common, are localized in comparatively small areas.

Trapped mice were brought into the laboratory, examined for warbles, and then placed in hardware-cloth cages kept over receptacles containing slightly moistened earth. When the study was begun, the *Cuterebra* larvae were permitted to emerge naturally, but after it was discovered that the mice sometimes attacked, killed, and ate the maggots while they were emerging, the maggots were extracted from the mice just as they started to leave. This was accomplished by gently squeezing the skin of the mouse from behind the inner (anterior) end of the maggot. After a minute or two the maggot would loosen its hold and fall out. The larval "cyst" was not walled like that of the ox warbles, but was just an area in which necrosis of the muscle tissue had occurred. A scab usually formed in from one to two weeks, although in some cases the wound persisted for a longer period, continuously discharging pus and serum. All mice were retained for proposed infection experiments. A thorough internal examination of 50 infected mice was made in an attempt to discover a possible path of larval migration in the host. However, larvae in all stages of development were found only under the skin.

When the larvae left the host they were placed on earth into which they burrowed almost immediately, and within two days puparia were formed at a depth of one and

[2] This high per cent of infection and the large number of parasites per host are not rare occurrences. According to Vorhies and Taylor (1940) *Cuterebra* species are the most conspicuous parasites of *Neotoma*. In 39 consecutive examinations of these rats made by Spencer in the Verde Valley, 24 were found to be infected (almost 62 per cent), and as many as four or five larvae were found in some rats. Females were found with nursing young, all three being infected. Gander (1929) found about 50 per cent of the woodrats he examined parasitized and Merriam (1889) found about 50 per cent of the chipmunks at the south end of Lake Champlain infected with these parasites. Bailey (Vorhies and Taylor) recorded that each of five woodrats taken near Cliff, New Mexico had from one to three in its throat while Cameron (1926) reports that a rat harbored 17 larvae. Mr. Everett W. Jameson, Jr., informed the author that during a few weeks of trapping at Point Abino, Ontario, in the summer of 1942, he found almost every deer mouse to be the host of one to four warble fly larvae which came to rest alongside the testes in the males. He found the mouse population in his trapping area very low in comparison with previous years.

one-half to five inches. It was noted that the puparia (with the enclosed pupae) were only about four-fifths as heavy and four-fifths as long as were the larvae before pupation. This must have resulted primarily from a loss of moisture and is probably an adaptation to prevent excessive freezing of the overwintering pupae. The above measurements were made from the records of thirteen larvae and the resulting puparia.

After measurements were taken, the puparia were placed in two pails of earth which were kept in an outdoor subterranean basement during the winter months.

FIG. 2. *Cuterebra* larvae in various stages of emergence from rodent host. Note that the posterior end leaves first. (Photograph by George McClellan Bradt.)

Thermograph records showed that the temperature of the soil in the pails varied from 25 to 41° F.

In mid-April, 1941, the pails were removed from the basement and kept outdoors. An outdoor cage, 7 · 5 · 5 feet, constructed of window screening on a wooden frame and without a bottom, was built to house the flies when they emerged. Another cage, $4 \times 4 \times 2\frac{1}{2}$ feet, constructed of sheet metal and with an open top, was filled with a layer of earth and nesting material and was stocked with ten mice. This cage, along

with the pails containing the pupae, was placed in the larger cage, the latter being partially covered with cheesecloth to simulate the mottled shade and sun of the woodlands. It was hoped that the emerging flies would have ample room to react normally in the presence of their host and that their method of oviposition would thereby be learned.

The first adult fly, a female, emerged May 31. When the fly was disturbed it made a loud buzzing noise, louder than, though distinctly different from that of a bumblebee. On June 1 a second fly, a male, emerged. The flies were quiet and it was usually difficult to find them since they remained well-hidden within a hollow log or on the underside of leaves. When the leaf upon which a fly was resting was picked up, the fly still did not move. Although they were clumsy on their feet, they were excellent flyers. At no time did the flies seem attracted to the mice, but they often were seen near the entrance of the mouse burrows. The male fly died in three days and the female disappeared after ten days, oviposition not having been observed.

Since so few flies had emerged, the remaining puparia were dug up and four of them were found to be empty. It is likely that the flies that had emerged from these puparia, as well as the one missing fly mentioned above, landed near the mice and were eaten. On August 21 another fly, a male, emerged and was immediately pinned.

In 1942 the author obtained a number of adult *Cuterebra peromysci* which emerged from puparia collected the previous year. The eggs removed from one dissected female, as well as those laid on twigs and paper by another, averaged 1.4 mm in length and 0.3 mm at the widest point. They displayed a very heavy chorion, a ventral groove, a sticky covering on the caudal end, and an operculum on the dorsal aspect of the cephalic end. Approximately 450 eggs were removed from the dissected female and the living fly deposited a like number. Sporadic egg-laying began two days after the emergence of the female, the fly ejecting one egg after another for a time, then moving about a bit and depositing no eggs, then laying one or two eggs, and occasionally refraining for long periods. About 45 hours were required for the deposition of all the eggs. The eggs never completely overlapped each other although several shingled masses were formed. When ovipositing on a twig, the female encircled it with her eggs.

DISCUSSION

The method by which *Cuterebra* larvae gain entrance into the host has been open to much conjecture. Some have assumed that the ventral groove of the egg indicates that the eggs definitely are deposited on the hair of the host, and that the operculum can only be opened by friction and moisture applied by the tongue of the host when licking its fur. It follows that the larvae which are taken into the mouth penetrate the tissues, migrate to the subcutis where they mature, and finally emerge through holes they produce in the skin of the host. Others modify this approach. They believe the eggs are laid on food plants of the host and that when the latter feeds, the eggs are also ingested. Friction and moisture in the mouth again cause the operculum to open and the rest of the development is the same as has already been described.

Moilliet (1943), and also Ferris (1920), however, have had the larvae emerge from eggs with no mechanical aid. Should the eggs be deposited on the host, the

tremendous numbers laid by *Cuterebra* species (more than 1000 eggs have been taken from one *Cuterebra horripilum*) would be unnecessary. Since the large number of eggs produced indicates that oviposition does not occur on the host, the first instar larvae must have a reduced chance of reaching a suitable host, and therefore a high mortality rate. Parker and Wells (1919), and Moilliet found eggs to remain viable for at least six months and Moilliet kept one unfed larva alive for ten days. Both of these investigators found the newly emerged larvae to be very active. These specializations seem unnecessary if the female deposits her eggs directly on the host animal.

Infection through the mouth precludes the possibility of infection of those animals that have not yet begun to feed. However, Mr. Ralph H. Smith, Game Research Investigator, New York Wildlife Research Center, found a six-day-old cottontail already parasitized (personal correspondence) and Spencer (Vorhies and Taylor, 1940), found female woodrats and their nursing young all infected.

It is not surprising to find that some of Parker and Wells' transfers of larvae to the mouths of prairie dogs were successful since there is no reason to suppose that larvae, that do happen to reach the mouth cavity, cannot penetrate the mouth tissue. However, it will be noted that over three-fourths of the larvae failed to become implanted and that some larvae were found dead in the host tissue. This might be due, in part, to the difficult migration the larvae would have to make before reaching their ultimate points of localization under the skin of the host.

The very heavy chorion of the *Cuterebra* eggs indicates that they are laid intact and that the enclosed larvae do not emerge for some time after oviposition. As indicated in the author's observations of adult *Cuterebra peromysci*, the flies of this group seem to be stealthy and usually never come in contact with the host. Townsend (1935) observed many of these flies in the wild and has seen them fly to rockpiles, small recesses in rocks, and wood or brushpiles that might have formed rodent habitations.

It seems probable that the eggs of *Cuterebra* species are deposited among the nests, burrows, or logs used by the hosts. The young larvae emerge intermittently, become attached at their posterior end, and sway up and back while awaiting a suitable host. When they come in contact with such an animal, they attach to it, perhaps move about over it, and burrow in when a satisfactory spot is reached. They continue their development in the subcutis of the host. Since definite cyst walls are not formed around the larvae, it is possible that the larvae may migrate a distance under the skin while feeding. They finally emerge through either the hole they formed when entering, or, if they migrated, through another hole produced elsewhere. Although this relationship of larva to host is supported by much circumstantial evidence, it cannot be proved definitely until additional observations have been made.

Another problem arising in the study of the cuterebrid parasites is the length of larval development in the host. Both Vorhies and Taylor, and Moilliet concur on this point, finding the larval period of *Cuterebra tenebrosa* Coq. to be from about three weeks to a little over a month. Scott and Snead (1942) were able to determine the infection period of a mouse-infecting species (probably *C. peromysci*) as approximately one month.

The pupal period of *C. tenebrosa* under laboratory conditions was found by Parker and Wells to be 47 days in one instance and 173 in another, while Moilliet found it to be about 11½ months for a pupa of the same species kept in an outdoor insectary. The pupal period of *C. peromysci*, the larvae of which were taken during the period from September to November by the author, was from 8 to 10½ months, while it was less than two and one-half months for one pupa, the larva of which was taken in mid-July. The large range of pupal period indicated in the records of Parker and Wells, and of the author, may be accounted for in part by the amount of food stored in the puparia, the moisture, temperature, and other environmental factors. Another consideration might be the time of year in which pupation occurred. The pupal stage may be short during mid-summer and very long if initiated during the fall and winter.

It is generally agreed that in North America *Cuterebra* species overwinter in the pupal stage. However, the heavy chorion of the eggs, the long period of incubation before larvae begin to emerge from the eggs (Moilliet found this period to be over a month under laboratory conditions), the ability of the eggs to remain viable for at least six months (Parker and Wells, and Moilliet) and to withstand sub-freezing temperatures (Moilliet) indicate the possibility that at least some species may also overwinter as eggs. It is likewise assumed that there is only one generation per year. However, the short pupal periods of two different species reared by Parker and Wells, and the author indicate that there might be an additional generation during the summer months. In both cases the flies developed from larvae that were taken early in the summer.

Lastly, the effects of these parasites on their hosts should be mentioned. Although in a few of the reported cases of *Cuterebra* infection the larvae emerged without having inflicted any apparent hardship on the host, most investigators have observed that infected rodents were awkward and therefore easy prey for predators. Scott and Snead found a correlation between the decrease in mouse populations and the peak of *Cuterebra* infection. Jameson, in his trapping of deer mice at Point Abino, Ontario, found the mouse populations greatly reduced in relation to what they had been in previous years but the mice he did find were almost all parasitized. Lindquist (1937) has shown that the warble, *Cuterebra buccata* Fab., was the principal predisposing cause of infection in cottontail rabbits in Uvalde, Texas, by the primary screwworm, *Cochliomyia americana* C. and P.

Dr. Kenneth Bogard (personal communication) removed a larva of *Cuterebra horripilum* from the neck of an eight-week old kitten and also one from a litter-mate. Both kittens were thin and unthrifty. Crushing of a *Cuterebra* larva in an attempt to remove it from under the skin of a dog caused symptoms like those of anaphylaxis (Cameron). Rabbit breeders have mentioned to the author that it was often necessary for them to kill some of their stock because of the large size of the wounds caused by these larvae. Moilliet found that infection with these parasites led to the death of many of his experimental rats and Parker and Wells found one of their three experimental prairie dogs seriously affected by a larva. The author had about 20 per cent of his infected mice die from unknown causes just at the time the larvae were emerging (this does not include those that died of *Salmonella* poisoning). It has been the experience of Cornell veterinarians that wounds in cats and dogs

caused by these larvae are dirtier and of a longer duration than those caused by other myiasis-producing DIPTERA. This might be accounted for by the lack of a definite cyst wall surrounding the larva, which condition would permit more easy infiltration of toxic materials into the surrounding tissues. The wounds do not start to heal for a long time, often remaining open for weeks with an accompanying discharge. Pyemia, with resulting death, sometimes occurs.

Cuterebra larvae also affect the food value of animals. Mr. A. C. Shaw, Assistant Regional Forester, Atlanta, Georgia, notes: "Infestations in the larval state have been noticed in this region on squirrels during the early fall hunting season. Natives refer to these as warbles, and usually infested squirrels are discarded as inedible" (personal communication).

These parasites do have a definite affect on rodent populations and must therefore be a factor in the control of rodent ectoparasites that act as vectors of important human and animal diseases. It would appear, therefore, that this group of myiasis-producing flies deserves greater attention than it has received in the past.

SUMMARY

Original and recorded observations in *Cuterebra* species are discussed from the following standpoints: (1) The method by which *Cuterebra* larvae gain entrance into their hosts, (2) oviposition, (3) length of larval development in the host, (4) length of the pupal period, (5) overwintering of the parasites, and (6) the effect of the larvae on their hosts.

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IN VITRO CULTIVATION OF TREMATODE METACERCARIAE FREE FROM MICROÖRGANISMS

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Cercariae of the strigeid trematode *Diplostomum flexicaudum* (Cort and Brooks, 1928) naturally develop into metacercariae in the eye lenses of many species of fishes (Ferguson and Hayford, 1941). Recent studies have shown that the metacercariae of this fluke will also grow normally in the lenses of frogs, turtles, chicks, ducklings, and laboratory mice, rats, guinea pigs and rabbits (Ferguson, 1942). This is a report of experiments in which it has been found that axenic* cercariae of *D. flexicaudum* will develop at room temperature into metacercariae when placed in culture media containing lens material from the eyes of vertebrates representing both cold- and warm-blooded groups.

MATERIALS AND METHODS

Cercariae used in these experiments came from laboratory-reared snails (*Lymnaea stagnalis*) infected with a strain of *D. flexicaudum* obtained through the courtesy of Dr. F. G. Wallace, University of Minnesota. The larval worms were allowed to emerge in half pint milk bottles containing a little aquarium water. For use in certain experiments they were concentrated to form a heavy suspension in water by slow speed centrifugation in special tubes elsewhere described (Ferguson, 1943).

The cercariae had to be free from contamination with microörganisms before they would survive and develop in cultures. This is in accord with observations made by various authors who have attempted to obtain development in vitro of parasitic worms of vertebrates (Ferguson, 1940). It has not been possible to obtain axenic cercariae for inoculation into cultures by washing the larval worms in sterile water, nor did they survive treatment with any one of several chemical sterilizing agents used at various dilutions. The cercariae were, however, freed from contamination as they penetrated a susceptible fish host and migrated to the eye for localization in the lens. Hundreds of larval worms were found in the lenses of blackhead minnows (*Pimephales promelas*) and fingerling rainbow trout (*Salmo irideus*) if a few of these fish were allowed to swim for about an hour in a small amount of water containing several thousand recently emerged cercariae. Fish so treated showed a heavy mortality, but this could be avoided if the organisms were concentrated as described above and were injected into the orbital space by means of a syringe needle inserted beneath the tissues surrounding the eyes. From this location large numbers of cercariae migrated into the eye and reached the lens within a few hours. This

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* The adjective *axenic* was introduced to denote a living organism that is free from all other demonstrable organisms (Baker and Ferguson, 1942, Proc. Soc. Exper. Biol. and Med. 51: 116). It is a more convenient term than expressions currently used, e.g., "animals free from microörganisms," "animals free from contaminating organisms," and "animals free from contaminants" and in meaning it is more correct than the adjectives "sterile" and "germ-free." Axenic organisms are defined as individuals of a species free from any life apart from that produced by their own protoplasm.

method of obtaining large numbers of cercariae in the lenses has also been used with *Lepomis macrochirus*, the bluegill sunfish, which acquired few or no larval worms even when exposed over several hours to thousands of cercariae in a small amount of water. A few dozen cercariae eventually reached the lenses of bluegills when large numbers were injected into the coelomic cavity.

In some experiments cercariae were allowed to reach the lenses of the above-mentioned fish, and then the lenses were removed aseptically and introduced directly into culture media where development occurred. In other experiments where uninfected fish lenses formed part of the culture medium, and in all experiments in which lens material from animals other than fishes was used, the axenic cercariae were first recovered from the lenses into which they had migrated. This recovery was accomplished as follows. A few hours after exposure to cercariae, the fish were killed and the lenses removed aseptically with sterile forceps through a cut in the cornea from eyes which had previously been painted with tincture of iodine and then flamed lightly with a gas burner. Several lenses were dropped into a tube containing Tyrode solution. Using bacteriological precautions the lenses were macerated in the test tube with a glass rod. This procedure liberated most of the cercariae. The lens material and cercariae were transferred in a pipette to a sterile centrifuge tube of Tyrode solution, where the larval worms were allowed to filter through stainless steel wire cloth (80×80 mesh per inch), thus separating themselves from most of the lens debris. The cercariae settled through the salt solution into the tapered end of the centrifuge tube where they were recovered in a pipette for inoculation into culture tubes.

By carefully following these procedures axenic cercariae were regularly obtained, and no microörganisms could be demonstrated when samples of these larval worms were inoculated into routine laboratory media under aerobic and anaerobic conditions.

All cultures were contained in small test tubes (13×100 mm). These tubes were stoppered with short cotton plugs over the surface of which a layer of sealing wax was applied in order to prevent evaporation during the several weeks required for the development of the metacercariae. All culture experiments were carried out at room temperature.

MEDIA AND GROWTH IN STERILE CULTURES

In early experiments recently infected lenses of bluegill sunfish that had been exposed to cercariae by one of the methods described above were added individually to the small test tubes containing varying amounts of one of the following liquid media: Tyrode solution, Tyrode solution plus chicken serum, Tyrode solution plus a special bakers' yeast extract, bovine aqueous humor, and bovine vitreous humor. A few tubes contained Tyrode solution that had been injected into the coelomic cavity of large bluegill sunfish and withdrawn after two hours. Similar methods were used by Weekers (1939) for obtaining a medium in which rabbit lenses would survive and remain transparent and intact.

Varying degrees of development of cercariae toward the metacercarial stage occurred in these different cultures with the exception of those containing either bovine aqueous or vitreous humor. In this connection it has been found that these larval trematodes will penetrate the eyes of blackhead minnows and young rainbow trout from which the lenses have been removed previously, but they survive for only about a day in the humors of the eye. In many of the cultures the lenses remained intact

and transparent for 2 or 3 weeks and the developing metacercariae could be observed in the culture tubes when the latter were held horizontally under a dissecting microscope.

Cultures containing only the infected lens and either one or two cubic centimeters of Tyrode solution yielded fully mature metacercariae after about 3 to 4 weeks. When axenic cercariae were added to cultures containing an uninfected sunfish lens the larval worms were able to penetrate the lens and develop into metacercariae. Every culture experiment did not yield metacercariae. In many tubes the cercariae would show a small amount of development and then die. Whether there were significant differences in the lenses is not known. However, it was found that cercariae never survived long or showed much development when infected rainbow trout lenses instead of those from bluegills were placed in cultures. Metacercariae developed in lenses that remained intact and transparent or became opaque while remaining intact, and also in tubes where the lenses had disintegrated within a few days to form a flocculent mass.

As many as 155 and in many cases between 75 and 100 metacercariae developed in a culture containing but a single sunfish lens. Where the number of larval worms in a culture was this large, they tended to vary in size from very small partially developed forms to fully mature and large metacercariae, even after the cultures had been held for over 7 weeks. When fewer metacercariae were present in a culture they were usually larger. Metacercariae developing in this medium have lived and remained fairly active in cultures that were held over 4 and 6 months respectively. Usually, however, the metacercariae began to die or became inactive and opaque two months or more after the cultures were initiated. This is in contrast to the survival of metacercariae for over 15 months, and perhaps years, in the lenses of fishes, the natural hosts.

Fully developed metacercariae have been obtained in cultures consisting of two cubic centimeters of Tyrode solution and the lens of a laboratory rat. Here axenic cercariae were introduced into the tubes after the lens had been added to the salt solution. No survival or growth of axenic cercariae occurred in cultures where the rat lens was replaced by a small piece of either a rabbit lens or bovine lens (pieces the size of or a little larger than sunfish lenses, which are 3–4 mm in diameter). In contrast to these results many large and active metacercariae normal in appearance have been obtained in cultures containing rabbit or bovine lens substance but where the Tyrode solution was replaced by frog Ringers solution.

When the cultures were first initiated the pH of the medium was usually about 7.8. After several weeks when the metacercariae were mature the pH of the media varied from slightly below 7.0 to 7.5.

The infectivity of metacercariae developing in many of the culture experiments has been tested by feeding large numbers of these larval worms imbedded in the bodies of small rainbow trout to laboratory-reared laughing gulls (*Larus atricilla*) or by injecting them directly into the duodenum of 2 day unfed baby chicks. Adults of *D. flexicaudum* have not yet been obtained.

Data concerning the axenic metacercariae developing in cultures are given in Table 1. For comparison, data on metacercariae growing in the eyes of both cold- and warm-blooded vertebrates are also given. Of prime interest in the table is the range in body size of the fixed metacercariae from the various sources. A con-

siderable size variation exists among the metacercariae in each collection. The size of the larval worms developing in culture is in some instances greater than that of metacercariae recovered from the eyes of living animals.

TABLE 1.—*Data, including range in body size, concerning metacercariae of* D. flexicaudum *developing in sterile cultures and naturally in the eyes of experimentally infected cold- and warm-blooded vertebrates*

Source of metacercariae	Period of time larval worms were present in cultures or in vertebrate eye	Condition of metacercariae before fixation	Range in body size* of 10 metacercariae after fixation†	
			Length	Width
Cultures	*Days*		*mm*	*mm*
1 ml Tyrodes + one sunfish lens	52	Active, normal in appearance	0.27–0.60	0.09–0.30
2 ml Tyrodes + one sunfish lens	52	ditto	0.27–0.57	0.09–0.25
2 ml Tyrodes + one rat lens	46	ditto or opaque and dead	0.15–0.28	0.07–0.12
2 ml frog Ringers + piece of rabbit lens	46	Active, normal in appearance	0.27–0.63	0.09–0.27
2 ml frog Ringers + piece of bovine lens	46	Active, but slightly opaque	0.22–0.49	0.09–0.16
Eye of vertebrate				
Bluegill sunfish (*L. macrochirus*) No. 1	70 +	Active, normal in appearance	0.31–0.60	0.18–0.31
Bluegill sunfish (*L. macrochirus*) No. 2	60 +	ditto	0.31–0.39	0.13–0.19
Frog (*Rana pipiens*)	57	ditto	0.25–0.35	0.13–0.17
Laboratory mouse	26	ditto	0.45–0.50	0.16–0.20
Laboratory rat	27	ditto	0.20–0.40	0.10–0.17

* Range in size of body of 10 cercariae recently emerged from snail host and fixed in hot alcohol-formalin-acetic solution was 0.10–0.18 mm in length and 0.03–0.04 mm in width.
† Fixation in hot alcohol-formalin-acetic solution.

DISCUSSION

Axenic cercariae of *D. flexicaudum* remain alive in Tyrode solution, without lens material, for only about one week and little or no development occurs. It can be concluded from the experiments that the lens material from the various sources must contain the principal substances necessary for the growth of the cercariae into metacercariae. The fact that development occurred in cultures containing lens materials from fish, rats, rabbits, and cattle shows that the chemical composition of the lens materials from the different vertebrate species must be rather similar. These results were not surprising after it had been determined that the cercariae could survive and develop in the lenses of living laboratory mice, rats, guinea pigs, and rabbits (Ferguson, 1943).

Here it should be mentioned that the lens proteins of more or less closely related vertebrates within a class are apparently similar (Markin and Kyes, 1939), while differences have been demonstrated in lens proteins of animals belonging to separate classes (Ecker and Pillemer, 1940). These chemical differences are apparently not great enough to make the lens substance from any of the particular animals employed in my experiments unsuitable nourishment for the developing metacercariae.

It is significant that metacercariae developed in fish and rat lenses that disintegrated or became opaque soon after being introduced into the cultures and in tubes containing pieces of rabbit and bovine lens. If development occurred only in intact lenses in a culture medium where they remained transparent it would have to be admitted that the larval worms required an environment similar to that found in tissue cultures. These findings are encouraging when one contemplates the problems involved in analyzing the culture medium to determine the specific nutritive requirements of these larval helminths.

The salt content of the medium is an important factor and apparently requires variation depending on the source of the lens substance. This is clearly shown by the fact that metacercariae developed in Tyrode solution plus either sunfish or rat lenses but failed to grow in Tyrode solution plus lenses from rainbow trout or pieces of rabbit or bovine lens. However, development occurred in cultures containing pieces of rabbit or bovine lens when frog Ringers solution was used instead of Tyrode solution. The necessity of determining the proper salt content in the medium is emphasized by Stoll (1940) in his sterile culture experiments with the nematode *Haemonchus contortus*, and Ferguson (1940) in describing the cultivation of axenic adults of the trematode *Posthodiplostomum minimum*.

The presence of a great many small partially developed metacercariae in cultures where the larval worms were present in large numbers was to be expected. Their failure to grow larger was probably due to several factors among which the smaller amount of food material, an unfavorable oxygen balance, and the accumulation of excessive amounts of waste products were likely important.

SUMMARY

Methods are described for obtaining the cercariae of the strigeid trematode *Diplostomum flexicaudum* free from microörganisms. When introduced into culture media consisting of either Tyrode solution or frog Ringer solution and lens materials from fish, rats, rabbits, or cattle, these axenic cercariae developed into metacercariae that were morphologically normal, but they failed to infect chicks or laughing gulls.

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PRELIMINARY STUDIES ON THE PHYSIOLOGY OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE). I. THE HATCHING OF THE EGGS UNDER STERILE CONDITIONS

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The study of the metabolism of the egg and larval stages of mosquitoes requires their culture under sterile conditions. This has been accomplished in a number of cases by sterilization of the surface of the eggs and their subsequent introduction into sterile media, but the varied methods employed by previous workers have given inconsistent results regarding the influence of living micro-organisms on the hatching of *Aedes aegypti* eggs. This study was undertaken to settle the point.

Atkin and Bacot (1917) recorded that the presence of bacteria or their products stimulated the hatching of the eggs of this mosquito. This stimulation was demonstrated to be apart from the alkalinity or acidity produced by bacterial growth. Living cultures of *Staphylococcus aureus* and *Bacillus coli communis* caused the immediate hatching of dormant eggs. Eggs also hatched in the autoclaved extracts of these bacteria. The filtrate of *Bacillus coli communis* was not effective in inducing the eggs to hatch. Living cultures of yeast were similar to bacteria in effecting the hatching of the eggs. Though they ran no tests on molds, observations in cases of breakdown in sterility indicated that they had a similar effect.

Roubaud and Colas-Belcour (1927) observed that in pure or only slightly impure water the eggs of *Stegomyia fasciatus* (*Aedes aegypti* L.) may lie dormant for months, but hatch in water rich in organic matter, and containing bacteria and yeasts. They also found them to hatch in living cultures of *Bacillus coli* and yeasts, and in filtered extracts of such cultures. These authors believed that soluble ferments, which are contributed by bacteria, are responsible for the hatching of the eggs. They found that the enzymes, pepsin, papain, trypsin, and tyrosinase seemed to cause the eggs to hatch. They were unable to induce hatching with the digestive or fermentative products of proteins, i.e., urea, glycocoll, alanine, indol, etc. Likewise, the acids and alkalies of fermentation (lactic acid, ammonia, carbon dioxide) failed to cause the eggs to hatch. Later Roubaud (1927) reported that the action of calcium hypochlorite simulated the action of the proteolytic enzymes in stimulating the eggs to hatch.

Hinman's results (1930) disagreed with those of the above authors. He found that the eggs of this species hatched as readily in sterile as in contaminated media. Barber (1928) also reported no indication in his experiments of any promotion of hatching by bacterial growth.

MacGregor (1929) described the normal development of *Aedes aegypti* from egg to adult on a sterile medium consisting of bread in distilled water. Hinman (1932) and Rozeboom (1935) attempted to repeat MacGregor's work, but succeeded only in producing development of the larvae to the second instar. Trager (1935) found that medium consisting of 0.5% Eli Lilly Liver Extract No. 343 in

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combination with a 1% suspension of either brewer's or baker's yeast was sufficient for the hatching of the eggs and the normal development of the larvae under sterile conditions.

MATERIALS AND METHODS

A colony of mosquitoes was maintained to furnish eggs for the tests. These eggs were laid on a filter paper disc partially lining the sides of a beaker about half full of distilled water (method of Trager, personal communication). Except where otherwise stated, the eggs for each test were collected during a twelve-hour period and were kept moist for twelve additional hours before sterilization and introduction into culture tubes.

A modification of Trager's (1935) technique was used in sterilizing the surface of the eggs. "Bowls" were made of Will Corporation's 10 mm circular cover glasses by flaming the edges until they turned under to form an oval-shaped bowl. At the time of sterilization the desired number of eggs was forced down through the surface film of water contained in the bowl. White's solution, as employed by Trager (1937), was used for the sterilization of the egg surface, since a comparative test of the effectiveness of this agent had shown it to be far more effective for this purpose than either hexyl resorcinol, seresan, or merthiolate. The sterilizing agent was run into a sterile petri dish and the bowl of eggs was sunk into the solution. The few eggs which were driven from the bowl by convection currents were returned with a capillary pipette. The cover was placed on the petri dish and left for the duration of the twenty-minute period of treatment. Forceps, well flamed, were used to transfer the bowl of eggs to a pyrex culture tube (19×150 mm) containing 10 ml of sterile distilled water. One of the eggs was then taken with a sterile capillary pipette and introduced into each of the culture tubes which contained 5 ml of media. Nutrient broth, sterilized by autoclaving, was used as a medium except in cases otherwise specifically stated. The value of nutrient broth for this purpose lies in its becoming cloudy with bacterial growth, thus serving as an indication of contamination. The cultures were incubated at 26° C to 29° C, and observations of the cultures were made at regular intervals of twelve hours.

EXPERIMENTAL DATA

Evidence that contamination of medium stimulates hatching.—In one test, 100% sterility of the egg surfaces was effected by the treatment of eighty-four eggs with the germicide. However, only one of these eggs hatched by the end of the fifth day of incubation. Thirty-eight of the sterile cultures were then exposed to contamination by removing the cotton plugs. Twenty-six of these contaminated tubes showed hatching by the end of the eighth day, while no further hatching occurred in the 46 tubes kept sterile.

It seemed possible that this apparent lack of harmony with the results of Trager (1935) may have been due to the media used, since Trager had allowed the eggs to hatch in a 0.5% solution of liver extract (Eli Lilly, Extract No. 343) instead of nutrient broth. To test this possibility, one sterile egg was placed into each of twenty tubes which contained 5 ml of 0.5% solution of liver extract (Eli Lilly). By the end of the fifth day of incubation only one of the eggs had hatched. Ten of the remaining tubes, in which no hatching had occurred, were then exposed to the air for

contamination. Eighty per cent hatching occurred in the contaminated tubes by the end of the eighth day, while no further hatching occurred in the sterile tubes.

Ten eggs which were taken at the end of the fifth day from sterile tubes of nutrient broth and introduced into ten tubes of autoclaved 1% suspension of brewer's yeast (Fleischmann's) in a 0.5% solution of liver extract also failed to hatch by the end of the eighth day, as did the ten check eggs left sterile in nutrient broth.

It thus appeared that a large proportion of the eggs of the particular strain of *Aedes aegypti* used in this study do not hatch in the absence of living micro-organisms, or their products, in either nutrient broth, liver extract, or in a combination of killed yeast and liver extract. Contamination with micro-organisms, however, seems to cause a relatively high percentage of hatching.

Effect of pure cultures of fungi.—Pure cultures of several species of micro-organisms were next tested for comparative effect on the hatching of eggs. It was thought that a comparison and contrasting of the properties of the organisms might enable one to determine which properties influence hatching. The eggs used in these tests had been lying dormant in sterile nutrient broth for five days. Some of these were left undisturbed as controls. Inspection of Table 1 reveals that all of the micro-organisms tested are capable of stimulating the hatching of the eggs. This effect is exerted for the most part during the first twenty-four hour period of fungal growth. The fact that the hatching of the eggs in the presence of *Sarcina lutea* occurred somewhat later than in other cases may be explained by the relatively slow growth rate of this organism.

TABLE 1.—*The effect of various known bacteria, yeast, and molds on the hatching of dormant eggs of* Aedes aegypti

Fungi	No. of eggs, 1 per tube	Number hatched					% hatched by 60th hour
		12 hrs.	24 hrs.	36 hrs.	48 hrs.	60 hrs.	
Bacillus subtilis	30	28	2	..	..	..	100
Escherichia coli	30	28	2	..	..	..	100
Staphlococcus aureus	30	22	3	0	0	0	83
S. albus	30	22	5	0	0	0	90
S. citreus	30	18	10	0	0	0	93
Sarcina lutea	30	0	11	13	1	1	87
Serratia marcescens	30	27	0	0	0	0	90
Pseudomonas aeruginosa	30	26	1	0	0	0	90
Saccharomyces cervisiae	30	15	4	0	0	0	63
Aspergillus niger	30	23	0	5	1	0	97
Penicillum sp.	30	10	16	0	0	0	87
Rhizopus nigricans	30	24	0	0	0	0	80
Fusarium moniliforme	30	25	2	0	0	0	90
Glomerella grossypii	30	14	12	0	0	0	87
Combined controls	120	0	0	0	0	0	00

Effect of crowding.—One essential difference between the technique described above and that used by Trager (1935) lies in the number of eggs placed for hatching in each culture tube. Trager placed from fifty to two hundred in each culture, whereas the eggs were placed singly in the above experiments. To test for the possible effect of crowding on hatching, therefore, various numbers of eggs were placed together in the culture tubes. Table 2 gives the number of eggs in each tube and the percentage of hatching which occurred in each case. It should be noted that the eggs in fifty-one of the tubes which contained fifty-three or more eggs were collected during a forty-eight hour period, and were dried for eight days prior to surface sterilization.

TABLE 2.—*The effect of crowding on the hatching of the eggs under sterile conditions*

Eggs per tube	No. of tubes	Total no. of eggs	Total no. hatched by 5th day	% hatched
1	113	113	7	6
2	21	42	9	21
3–6	24	98	18	19
7–11	9	68	31	47
14–23	5	97	50	52
37–51	2	88	46	53
53	1	53	44	83
58	1	58	47	83
63	1	63	53	84
64	1	64	51	80
74	1	74	44	59
119	1	119	87	73

Thus it appears that the crowding of the eggs under sterile conditions produces a higher percentage of hatching than occurs when only one egg is contained in the culture tube, but still not as high a percentage as is produced by the concomitant growth of micro-organisms (Table 1).

The way in which the growth of micro-organisms and the simple crowding of the eggs promote hatching of the eggs remains an enigma. Two possibilities which seem to merit further consideration as factors involved in the initiation of the hatching process are the possible increase in the permeability of the egg wall to water and the change in the oxidation-reduction potential of the medium. Further studies regarding these possibilities are planned.

SUMMARY

1. White's solution as used by Trager (1937) was found to be 100% effective in the surface-sterilization of the eggs of *Aedes aegypti*.

2. *Aedes aegypti* which were cultured singly in five ml of sterile media (nutrient broth, or liver extract and killed yeast) rarely hatched.

3. Pure cultures of the following heterotrophic fungi were found to stimulate the hatching of dormant eggs: *Escherichia coli; Sarcina lutea; Pseudomonas aeruginosa; Serratia marcescens; Saccharomyces cervisiae; Aspergillus niger; Penicillum* sp.; *Rhizopus nigricans; Fusarium moniliforme; Glomerella grossypii.* Since all of these organisms produced a relatively high percentage of hatching in the eggs, it seems that this property is generally distributed among heterotrophic fungi. This substantiates the observations by Atkin and Bacot (1917) and Roubaud and Colas-Belcour (1927).

4. When eggs were crowded together in the cultures a relatively large percentage hatched, though not as large a percentage as when micro-organisms were introduced. This stimulation of hatching due to simple crowding may possibly explain why Barber (1928), MacGregor (1929), Hinman (1930), Rozeboom (1935) and Trager (1935, 1937) did not discover any relationship between the presence of living micro-organisms and the hatching of *Aedes aegypti* eggs.

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FLOWERS AS A SUGGESTED SOURCE OF MOSQUITOES DURING ENCEPHALITIS STUDIES, AND INCIDENTAL MOSQUITO RECORDS IN THE DAKOTAS IN 1941*

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Incident to late season studies made in North and South Dakota in connection with the 1941 outbreak of encephalitis, it was found that numbers of certain species of mosquitoes desired for tests for encephalitis virus could be obtained as readily from flowers as by general collecting from animals, roosts, and the like. The observations are reported as suggestive of the possible importance of flowers as a supplemental source of mosquitoes for test use.

It was presumed that most flower-visiting mosquitoes would be males and recently emerged females of little value as possible virus carriers because mostly unfed. Therefore, it was somewhat of a surprise to discover that certain flowers such as goldenrod (*Solidago*) were visited extensively, not only by males and young females, but also by females with blood meals in various states of digestion and even by some with abdomens swollen with eggs. Visitation was both diurnal and nocturnal. Among the species collected from goldenrod were *Aedes vexans, spencerii,* and *campestris, Culex tarsalis* and *territans,* and *Theobaldia inornata.*

It was also noted on several occasions in the late afternoon, that, unless there was a freshly emerged "crop" of *Aedes* due to recent rains, the bulk of the females (including the older ones that were more desirable from a test-standpoint) actually seemed to prefer the goldenrod blossoms to attacks on persons nearby. This facilitated collecting with an aspirator. Even in the late part of the season as the night temperatures approached freezing, the insects could still be taken by flashlight on the younger blossoms. This was also true in wet, cool weather, when collections elsewhere were mostly discouraging. For example, 3♂, 2♀ of *C. tarsalis*, 3♀ of *T. inornata* and 2♀ *A. vexans* were taken on goldenrod between 10:30 and 11:00 PM, September 9, at Webster, South Dakota, after a drop to 38° F from an afternoon temperature of 64° F.

Aedes spencerii, C. tarsalis, T. inornata, and *A. vexans* were found on flowers in that order of abundance, the females outnumbering the males. Among the important biters encountered in the area, only the anophelines were conspicuously

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absent. Only one female each of *A. maculipennis* at Rugby, North Dakota, and *A. campestris* at Pelican Rapids, Minnesota, were found on flowers.

Among incidental observations, it also may be mentioned that *A. maculipennis* was found in some abundance (not on flowers) in certain localities in central and eastern North Dakota, and in northeastern South Dakota. *A. punctipennis* was less common, but of special interest was the observed occurrence of *A. quadrimaculatus* at Valley City. One of two specimens seen was captured. The identification was confirmed by Dr. T. H. G. Aitken. The farthest previous northwest record of this important southern malarial vector is for eastern Minnesota, so this observation extends the distribution for a considerable distance into the Northern Plains area.

Among other observations of interest was the taking of a freshly fed specimen each of *A. maculipennis* and *C. tarsalis* in a chicken house at Towner, N. Dak. Several *C. pipiens* were captured at Waubay and Brookings, S. Dak., while *C. territans* as well as *C. tarsalis* were found breeding at several locations in North Dakota west to the vicinity of Rugby. In the earlier samples, *Aedes vexans* and *A. dorsalis* were the most abundant mosquitoes taken on domestic stock, but a mid-September rain resulted in emergence in countless numbers of a late brood of *A. spencerii* which then became the dominant pest of man and animals by September 22. This observation of a late summer brood (or at least a delayed hatching) may be of some consequence since both *A. idahoensis* and *A. spencerii* (which Matheson suggests as possibly the same) are reported in the literature as having only one annual spring brood which hatches from eggs laid the previous year, but the adults are reported to persist through the season into September. Specimens of both sexes were submitted to Dr. W. B. Owen who confirmed their identification.

LARVAL TREMATODES OF NORTHWEST IOWA. I. NINE NEW XIPHIDIOCERCARIAE[1]

F. G. Brooks

Studies on larval trematodes of the Okoboji region pursued by the writer during the summers of 1940, 1941, and 1942 have revealed a number of new forms. In the present paper nine stylet cercariae will be described.

METHODS OF STUDY

Snails were collected from the lakes, sloughs, and kettleholes that abound in the region. The usual procedure of isolating the snails in half-pint milk bottles was used. The cercariae were studied alive, both unstained and stained with various intra-vitam dyes of which neutral red and Nile blue sulfate proved to be most useful.

Measurements were taken on living larvae and on specimens killed in several ways. The method of heating them in a drop of water on a slide without a cover-slip seemed to produce results most in keeping with the measurements on the living larvae. The slide would be laid on an electric hotplate for five seconds. A second method was to centrifuge the contents of the milk bottle and, after taking up the material from the bottom of the tube with a pipette, squirting it into hot 10% formalin. This caused the cercariae to shrink considerably, but gave rather uniform results. For some forms both methods for killing were used, especially when it expedited the comparison with related cercariae. Averages of from ten to twenty measurements were usually taken.

The writer has been impressed with the uniformity of the dimensions of the stylets of the various species of xiphidiocercariae studied. He believes that greater use can be made both of the shape and length of the stylet in describing and in identifying cercariae of this group. He found no significant aberration in shape among the various specimens of any species studied. The fluctuation in length did not exceed one micron and seldom was any difference noticeable beyond the limitations of the ordinary micrometer. In this study, complete dependence was not placed on either the length or the shape of the stylet in distinguishing species, but such differences did corroborate determinations made on other grounds.

Cercaria lajeae n. sp.
(Figs. 1, 2)

Specific diagnosis: Xiphidiocercaria of Armatae group characterized by narrow body and conspicuous cornuae which nearly encircle acetabulum. Stylet needle-shaped with reenforced sides and basal bulb; 0.024 mm in length. Seven large penetration glands on each side anterior and lateral to acetabulum. Prepharynx shorter than pharynx; ceca short. Excretory bladder with bulbous posterior chamber and large cornuated anterior vesicle which nearly surrounds acetabulum; small granules (2–5 μ) found in both vesicles; posterior vesicle empties at base of tail through excretory canal equipped with heavy sphincter muscle; flame cells not visible. Cystogenous glands conspicuous; large glands in rows along lateral edges; clusters of small glands in region of pharynx. Oil-like refractile globules present in modern quantity. Body covered with fairly heavy spines in diamond cross-hatch pattern; spines heavier at anterior end.

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[1] From the Iowa Lakeside Laboratory and Cornell College.

The writer is indebted to Professor T. C. Stephens of Morningside College, a member of the staff of Iowa Lakeside Laboratory, for the identification of the snails mentioned in this paper.

Measurements: Living cercariae range from 0.510 × 0.065 mm when fully extended, to 0.180 × 0.160 mm when contracted. Tail length ranges from 0.425 mm to 0.120 mm. Average measurements in millimeters of emerged living cercariae moderately distended and of emerged cercariae killed with heat are:

	Living	Killed with heat
Body length	0.330	0.391
Body width	0.140	0.147
Tail length	0.370	0.340
Tail width	0.034	0.048
Oral sucker length	0.061	0.066
Oral sucker width	0.063	0.076
Acetabulum diameter	0.068	0.074
Stylet length	0.024	0.024
Stylet width	0.005	0.005

Precercarial stage: C. lajeae develops in yellow sporocysts that cannot be separated from tissue of digestive gland of snail.

Host and Locality: Found infecting *Physella elliptica* Lea collected from kettlehole five miles southwest of Lakeside Laboratory.

C. lajeae has many points in common with *C. brevicaeca* Cort, 1915, *C. ramonae* McCoy, 1928, *C.* of *Renifer kansenses* (Crow, 1913) described by McCoy, 1928, *C.* of *Lechriorchis primus* Talbot, 1933, *C.* of *Renifer aniarum* Byrd, 1935. *C. lajeae* differs significantly from all these forms in the shape of the stylet, by having fewer penetration glands and in having a muscular excretory canal. Since all cercariae of the genus *Renifer* whose life histories have been completed have an excretory bladder whose cornuae nearly surround the acetabulum, it is very probable that *C. lajeae* will be found to belong to this genus.

Cercaria kingi n. sp.[2]
(Figs. 3–5)

Specific diagnosis: An ornate xiphidiocercaria of Prima group. Tail short and stubby with dorso-ventral finfold. Stylet needle-shaped, 0.039 mm long; no basal bulb. Five pairs of penetration glands anterior and lateral to acetabulum; first two coarsely granular. Prepharynx nearly as long as esophagus; ceca extend to region of bladder. Excretory bladder with bulbous posterior chamber and v-shaped anterior vesicle; common duct of collecting tubes received terminally by cornuae; abundant cystogenous glands make determination of flame cell pattern impossible. Small, closely-set spines cover body; spines heavier at anterior end; protruding bristles (0.009 mm long) sparsely scattered over body.

Measurements: Body is distensible, attaining maximum length of 0.370 mm; contracted length 0.155 mm. Average dimensions of living cercariae and of those killed in 10% hot formalin:

	Living	Killed in hot 10% formalin
Body length	0.270	0.240
Body width	0.125	0.110
Tail length	0.160	0.137
Tail width	0.062	0.056
Oral sucker diameter	0.071	0.062
Acetabulum diameter	0.041	0.042
Stylet length	0.039	0.039
Stylet width	0.005	0.005
Greatest width of finfold	0.019	....

Host and Locality: Found as parasites of *Stagnicola umbrosa* Say collected 1940 from Manhattan Slough, two miles north of Lakeside Laboratory.

Lühe (1909) created the Ornatae group of xiphidiocercariae to include all stylets with a caudal finfold. Sewell (1922) subdivided this unnatural group and included in his Prima division those median-sized forms in which the acetabulum is

[2] Named in honor of Professor Robert L. King.

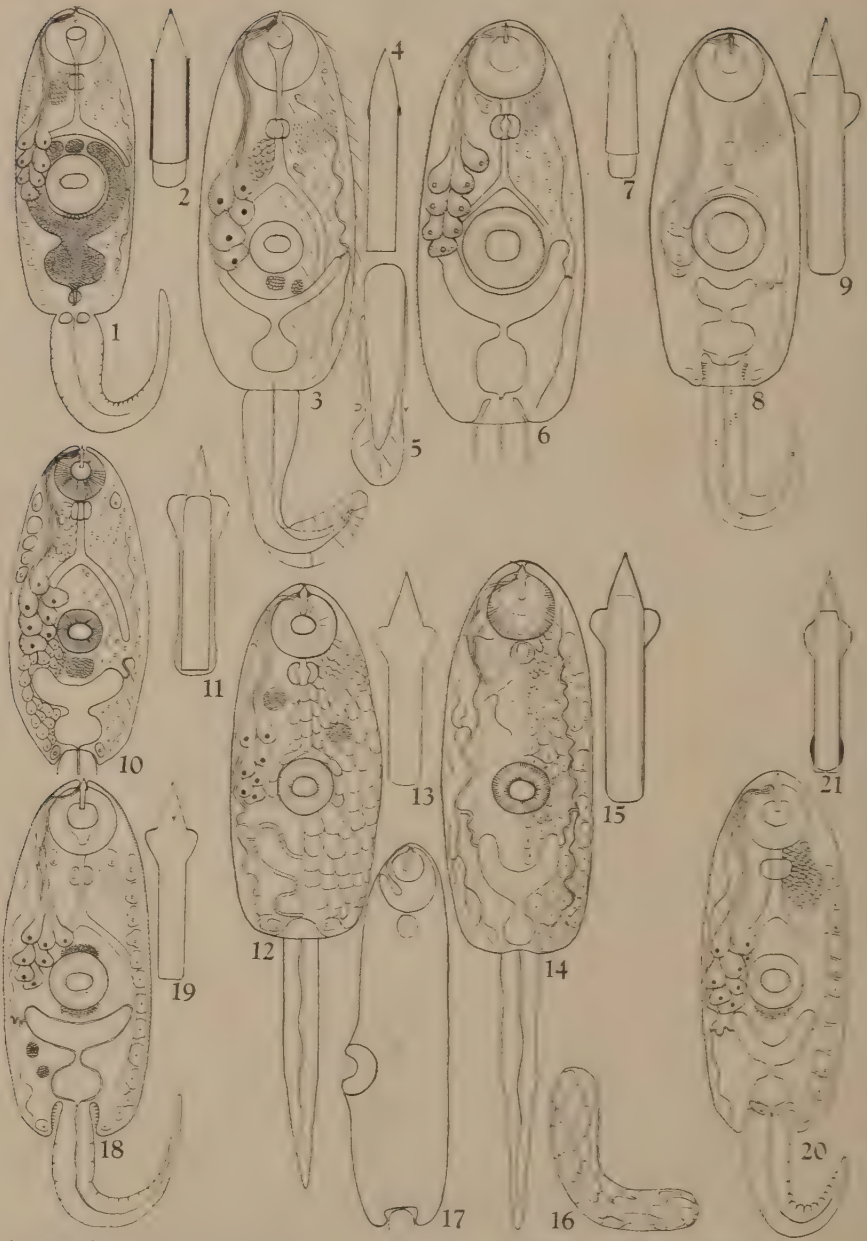

FIG. 1. *Cercaria lajeae* n. sp.
FIG. 2. Stylet of *Cercaria lajeae*.
FIG. 3. *Cercaria kingi* n. sp.
FIG. 4. Stylet of *Cercaria kingi*.
FIG. 5. Lateral view of tail, *Cercaria kingi*.
FIG. 6. *Cercaria diamondi* n. sp.
FIG. 7. Stylet of *Cercaria diamondi*.
FIG. 8. *Cercaria pili* n. sp.
FIG. 9. Stylet of *Cercaria pili*.
FIG. 10. *Cercaria conniae* n. sp.

smaller than the oral sucker; in which the tail is shorter than the body and has a dorso-ventral finfold of which the ventral portion extends farther forward; which have a prepharynx and pharynx and ceca reaching beyond the acetabulum; which have four or five penetration glands; which have an oval-shaped excretory bladder with cornuae; and which develop in oval or sack-shaped sporocysts.

C. kingi clearly belongs to the Prima group. Other members of this group are: *C. prima* Ssinitsin, 1905; *C. ornata* LaValette, 1855; *Cercariae indicae XXIV* and *XXVIII* Sewell, 1922; *C. longistyla* McCoy, 1929; *C. exilis* Brooks, 1930; *C. Pneumonoeces medioplexus* and *C. Pneumobites parviplexus* Krull, 1931; and *C. merchanti* Rankin, 1939. Of these, this new form most resembles *C. longistyla* but differs from it significantly in the length and shape of the stylet.

Cercaria diamondi n. sp.
(Figs. 6, 7)

Specific diagnosis: Xiphidiocercaria of Armata group. Stylet without shoulders and with basal bulb; stylet proper 0.019 mm long, bulb 0.004 mm long, slightly narrower than base of stylet proper. Eight pairs of penetration glands anterior-lateral to acetabulum. Excretory bladder with bulbous posterior chamber and v-shaped anterior vesicle; common duct from collecting tubes received subterminally. Cystogenous glands conspicuous. Refractile globules present in moderate amount. Body covered with fine closely-set spines.

Measurements: Average measurements in millimeters of emerged cercariae killed with heat:

Body length	0.418	Pharynx length	0.023
Body width	0.160	Pharynx width	0.032
Tail length	0.337	Middle acetabulum to posterior end	0.169
Tail width	0.039	Stylet length without bulb	0.019
Oral sucker length	0.063	Stylet width at base	0.004
Oral sucker width	0.071	Bulb length	0.004
Acetabulum diameter	0.071	Bulb width	0.003
Prepharynx	0.025		

Precercarial stage: Cercariae occur in delicate sporocysts with thin walls which cannot be separated from tissue of digestive gland of snail host.

Host and Locality: Obtained from two snails of collection of 480 specimens of *Physella elliptica* Lea collected 1942 at Diamond Lake, seven miles north of Lakeside Laboratory.

Activity.—C. diamondi is very active in water and continues the writhing motion characteristic of its group for a considerable time under a coverslip.

The "oily" globules and opaque cystogenous and parenchymous cells hide many finer structures. Globules of various sizes range from tiny to ovoid globules 0.009 mm long. All penetration glands stain with acid stain and are drained by four pairs of ducts whose openings can sometimes be seen at the front of the oral sucker on either side of the stylet. An occasional cercaria shows a large cell that stains heavily with neutral red near the oral sucker. The stylet lacks shoulders but shows a slight refraction of light where its sides start to taper. The basal bulb seems to be a continuation of the core of the stylet proper. In most larvae, one side of the stylet was nearly a micron longer than the other side. The stylets of the cercariae from the second snail were a micron longer than those from the first snail, but there was not that much range in the length of the stylets from either snail.

FIG. 11. Stylet of *Cercaria conniae.*
FIG. 12. *Cercaria argenti* n. sp.
FIG. 13. Stylet of *Cercaria argenti.*
FIG. 14. *Cercaria dorotti* n. sp.
FIG. 15. Stylet of *Cercaria dorotti.*
FIG. 16. Sporocyst of *Cercaria dorotti.*
FIG. 17. Lateral view of *Cercaria dorotti.*
FIG. 18. *Cercaria nolfi* n. sp.
FIG. 19. Stylet of *Cercaria nolfi.*
FIG. 20. *Cercaria aalbui* n. sp.
FIG. 21. Stylet of *Cercaria aalbui.*

C. diamondi resembles *C. holthauseni* Rankin, 1939, and *C. tricystica* E. L. Miller, 1935, in the number of penetration glands, type of the excretory bladder, length of stylet and in having a stylet bulb. It differs from both, however, in that the stylet is of different shape. It would seem that Rankin's form has less apparent cystogenous glands and refractile globules because he does not mention them and was able to work out the flame cell pattern. Miller's cercaria is considerably smaller than the present form.

Cercaria pili n. sp.
(Figs. 8, 9)

Specific diagnosis: Xiphidiocercaria of Armatae group. Stylet 0.020 mm long, javelin-shaped; no basal bulb. Pharynx situated more than half way between oral sucker and fork of esophagus; ceca short. Three pairs of penetration glands—two lateral to acetabulum and one slightly posterior; three ducts from penetration glands to point of stylet. Excretory bladder consists of bulbous posterior chamber and cornuated anterior vesicle which receives the common ducts from the anterior and posterior collecting tubes subterminally. Caudal receptacle large, muscular, caudal sinuses spined. Fine spines thickly set over entire body in diamond cross-hatch pattern.

Measurements: Average measurements in millimeters of emerged cercariae killed with heat:

Body length	0.195	Oral sucker width	0.038
Body width	0.065	Acetabulum diameter	0.032
Tail length	0.128	Stylet length	0.020
Tail width	0.032	Stylet width through shaft	0.004
Oral sucker length	0.041	Stylet width across shoulders	0.006

Precercarial stage: Produced in sporocysts that are nearly white, but with flecks of yellow pigment. Thin walled, but separate rather easily from tissue of digestive gland. Nearly uniform in diameter, but variable in length; larger ones measured 0.920 long by 0.130 mm wide.

Host and Locality: Found as parasites of *Helisoma trivolvis* Say collected at Hotte's Lake, ten miles northeast of Lakeside Laboratory.

Activity.—The mature cercariae leave the snail in the early morning and are no longer found swimming at noon. They are very active in the water and on a slide they use their tails more than other forms studied.

The body contains no refractile globules, but slightly refractive inclusions are at irregular intervals along the tail. Penetration glands are easily detected, with coarse, granular material. The stylet is held pointing downward at nearly a right angle and is large for the size of the cercaria. The cystogenous glands and parenchymous cells hide the smaller structures of the excretory system, making even the collecting tubes hard to see. The anterior vesicle is not often found to be filled in mature forms. The caudal receptacle is a characteristic feature. Its strong walls hold its shape after the tail is lost and at first glance might be mistaken for the bladder.

The form most resembling *C. pili* is *C. tridena* Miller, 1936. Their size, length of stylet, nature of larger excretory organs and number of penetration glands are similar or essentially so. Essential differences are found in the position of the pharynx, color of the sporocysts and in the fact that *C. tridena* has its caudal pockets reduced and these pockets are without spines in their walls.

Cercaria conniae n. sp.
(Figs. 10, 11)

Specific diagnosis: Xiphidiocercaria of the Armatae group. Stylet javelin-shaped, 0.029 mm long; shaft reenforced at base, no basal bulb. Prepharynx practically absent, pharynx well developed; esophagus forks well above acetabulum; ceca do not extend beyond posterior of

acetabulum. Seven pairs of penetration glands anterior and lateral to acetabulum drained by four pairs of ducts. Bladder with bulbous posterior chamber and v-shaped anterior vesicle; pair of short tubules leave cornuae subterminally to receive anterior and posterior collecting tubes. Dense cystogenous glands and abundant large oil-like globules obscure flame cells. Body spines short and sparsely distributed.

Measurements: Average dimensions in millimeters of moderately distended living cercariae and of cercariae killed in hot 10% formalin:

	Living	Killed in hot 10% formalin
Body length	0.270	0.158
Body width	0.128	0.092
Tail length	0.115	0.094
Tail width	0.040	0.026
Oral sucker, diameter	0.046	0.041
Acetabulum, diameter	0.036	0.029
Distance acetabulum to posterior end	....	0.045
Diameter larger oil-like globules	0.006	....
Stylet length	0.029	0.029
Stylet width across shaft	0.004	0.004
Stylet width across shoulder	0.007	0.007

Precercarial stage: Development occurs in long, tube-like sporocysts with small amount of golden pigment in walls.

Host and Locality: Found as parasites of *Stagnicola palustris elodes* Say collected 1940 from West Silver Lake Bog, ten miles northwest of Lakeside Laboratory.

Since this and the next four closely related cercariae seem to form a natural subgroup, their taxonomic relations will be discussed after they have all been described.

Cercaria argenti n. sp.
(Figs. 12, 13)

Specific diagnosis: Xiphidiocercaria of Armatae group. Stylet javelin-shaped with large shoulders; 0.032 mm long; no basal bulb. Body well supplied with cystogenous glands which give whitish luster. Refractile globules present. Seven pairs of penetration glands anterior and lateral to acetabulum. Prepharynx very short, not visible when larva is not extended. Esophagus and ceca difficult to distinguish. Excretory system consists of posterior vesicle which joins v-shaped anterior vesicle by narrow canal; both vesicles and canal with thick walls; main collecting tubes join cornuae subterminally. Very fine spines form diamond cross-hatch pattern over entire body, more conspicuous on ventral side and at anterior end. Small spines in caudal sinuses.

Measurements: Average measurements in millimeters of emerged living cercariae and of those killed with heat:

	Living	Killed with heat
Body length	0.340	0.315
Body width	0.144	0.124
Tail length	0.186	0.178
Tail width	0.038	0.035
Oral sucker length	0.068	0.062
Oral sucker width	0.066	0.060
Acetabulum diameter	0.049	0.047
Pharynx length	....	0.022
Pharynx width	....	0.020
Stylet length	....	0.032
Stylet width across shaft	0.005	0.005
Stylet width across shoulder	0.008	0.008

Precercarial stage: Sporocysts are short, thin-walled structures that do not separate readily from tissue of digestive gland.

Host and Locality: Cercaria argenti was found in 1940 infecting *Stagnicola palustris elodes* Say collected from West Silver Lake Bog, and, in 1942, in ten snails from collection of 620 of *Stagnicola umbrosa* Say collected from Stony Lake, eleven miles west of Lakeside Laboratory.

This cercaria is characterized by a great abundance of cystogenous glands which make most other structures difficult to distinguish. These glands are most dense on the ventral side. Refractile globules are present, but small and not so abundant as to hide other structures. The esophagus and ceca are effectively hidden by rows of small glands. The seven penetration glands are smaller than the average for closely related forms. Four ducts lead from these glands and empty near the base of the shoulders of the stylet. Two specimens contained round, glandular bodies which stood out quite distinctly from the surrounding structures. In each case one was located on the right side of the body just in front of the acetabulum while the other was on the left, farther front and more to the side. The cercariae react positively to light. They are vigorous; not only do they swim actively when free in water, but they maintain their characteristic "measuring worm" movement for a longer time than is usual for the other stylets after they have been placed on a slide.

It is worthy of note that the stylets of several hundred cercariae from eleven different snails of two species did not vary as much as a micron in length.

Cercaria dorotti n. sp.
(Figs. 14–17)

Specific diagnosis: Xiphidiocercaria of Armatae group characterized by very dense cystogenous glands. Stylet javelin-shaped; 0.034 mm long; no basal bulb. Penetration glands not determined, but five ducts can be seen to empty at base of stylet. Prepharynx very short; esophagus and ceca not seen in emerged specimens. Excretory bladder consists of bulbous posterior chamber and v-shaped anterior vesicle which receives common duct from collecting tubes subterminally. Very small refractile globules present, located principally at edges of cystogenous glands. Body covered by fine spines arranged in diamond cross-hatch pattern.

Measurements: Average measurements in millimeters of emerged living cercariae of medium contraction, of specimens killed by heat, and of those killed in hot 10% formalin:

	Living	Killed with heat	Killed in 10% formalin
Body length	0.280	0.314	0.237
Body width	0.115	0.120	0.102
Tail length	0.184	0.192	0.124
Tail width	0.034	0.034	0.031
Oral sucker length	0.066	0.058	0.048
Oral sucker width	0.066	0.060	0.049
Acetabulum diameter	0.045	0.041	0.033
Pharynx length	0.027	0.020	0.018
Pharynx width	0.021	0.024	0.018
Stylet length	0.034	0.034	0.034
Stylet width through shaft	0.005	0.005	0.005
Stylet width across shoulder	0.008	0.008	0.008

Precercarial stage: Sporocysts are small, orange-colored, sausage-shaped structures with blunt, rounded ends. Thickly packed in digestive gland of host from which they did not separate readily. Typical sporocysts measured from 0.9 to 1.2 mm in length and 0.170 to 0.210 mm in width.

Host and Locality: Found in single snail of *Stagnicola umbrosa* Say collected 1942 from Manhattan Slough, two miles north of Lakeside Laboratory.

Activity.—These cercariae emerge in the morning and spend several hours swimming actively. They are usually seen in swarms which may be found in any part of the bottle. They are neither attracted nor repelled by light of moderate intensity.

A study of immature cercariae revealed certain structures that were entirely hidden by the dense cystogenous glands of emerged specimens. The penetration

glands were found to be about the same size as the cystogenous glands, therefore, small, and at least five in number on each side. The esophagus was found to fork well above the acetabulum and the ceca to extend to the region of the bladder. A few immature cercariae showed the collecting tubes of the excretory system and their accessory branches very plainly. Each tube had three accessory tubules each of which gave off three capillary tubules. Thus there are evidently eighteen flame cells on each side, though none were seen. An immature cercaria studied in side view showed three nearly spherical bodies which were taken to be parts of the reproductive primordium and are shown in Fig. 17. Two bodies posterior to the acetabulum are evidently the testes while a large body above the anterior edge of the acetabulum may be either the primordium of the metraterm or of the cirrus or of both those structures.

Cercaria nolfi n. sp.[3]
(Figs. 18, 19)

Specific diagnosis: Xiphidiocercaria of Armatae group made opaque by abundant cystogenous glands and refractile globules. Stylet javelin-shaped with strong shoulders, 0.034 mm long; no basal bulb. Seven or eight penetration glands located anterior and lateral to acetabulum. Prepharynx short; esophagus forks short distance above acetabulum; ceca extend to bladder. Excretory bladder consists of bulbous posterior chamber and v-shaped anterior vesicle. Row of about twelve large cystogenous glands extends along each lateral edge of larva and smaller glands fill in solidly between; of these, a pair in each posterior lappet and at posterior-lateral edge of oral sucker are larger and more apparent. Body covered with fine spines in diamond cross-hatch pattern. Spines present in caudal sinuses. Reproductive fundament consists of cells in front of and behind acetabulum and two groups of cells lateral to opening between anterior and posterior vesicles.

Measurements: Average measurements in millimeters of emerged cercariae killed with heat:

Body length	0.328	Prepharynx	0.022
Body width	0.136	Pharynx length	0.024
Tail length	0.185	Pharynx width	0.027
Tail width	0.039	Stylet length	0.034
Oral sucker diameter	0.063	Stylet width through shaft	0.005
Acetabulum	0.051	Stylet width through shoulders	0.008

Precercarial stage: Sporocysts are long, orange-colored structures occurring in tangled masses. They are 0.350 mm in diameter and exceed one millimeter in length.

Host and Locality: Found as parasites of *Stagnicola palustris elodes* Say collected 1941 at West Silver Lake Bog and of *Stagnicola umbrosa* Say from Stony Lake.

Cercaria aalbui n. sp.
(Figs. 20, 21)

Specific diagnosis: Xiphidiocercaria of Armatae group. Stylet javelin-shaped but with slight ringed enlargement near posterior end; no basal bulb; 0.038 mm in length. Eight penetration glands anterior and lateral to acetabulum empty through four ducts which tend to become bulbous near oral sucker. Prepharynx short, pharynx broad, ceca cannot be distinguished below acetabulum. Excretory bladder with bulbous posterior chamber and v-shaped anterior vesicle whose cornuae receive common ducts from collecting tubes subterminally. Row of about twelve large cystogenous glands along each lateral edge; other glands of smaller size abundant especially in esophageal region; a gland in each posterior lappet and at each posterior-lateral edge of oral sucker are more noticeable than others. Refractile globules large and abundant, more numerous on dorsal side. Body covered by fine spines in diamond cross-hatch pattern, heavier toward anterior end; no spines in caudal sinuses.

Measurements: Living cercariae vary from 0.408×0.054 mm well extended to 0.170×0.140 mm contracted. Tail varies from 0.250 mm in length extended to 0.092 mm contracted. Average measurements in millimeters of emerged living cercariae and of those killed by heat:

[3] This species is named in honor of Professor L. O. Nolf.

	Living	Killed with heat
Body length	0.348	0.337
Body width	0.152	0.135
Tail length	0.216	0.225
Tail width near base	0.040	0.038
Oral sucker length	0.058	0.065
Oral sucker width	0.063	0.070
Acetabulum diameter	0.044	0.052
Pharynx length	0.018	....
Pharynx width	0.021	....
Stylet length	0.034	0.034
Stylet width across shaft	0.005	0.005
Stylet width across shoulders	0.007	0.007

Precercarial stage: Long yellow sporocysts occur in tight, tangled masses; old sporocysts become rusty colored; typical size of sporocyst 1.2 mm long by 0.27 mm in diameter.

Hosts and Locality: C. aalbui was found infecting numerous specimens of *Stagnicola palustris elodes* Say from several collecting grounds in 1940, 1941, and 1942; from *S. umbrosa* Say collected from Green Bay Bog in 1940, and from same species collected from Stony Lake in 1942.

Activity.—The swimming movements and behavior under a cover slip were typical for the group except that the tail of this cercaria seems to have adhesive qualities and the larva uses it for sticking to the substratum or any objects it touches. No glands were found to account for its adhesiveness. Martin, 1939, speaks of the cercaria of *Stephanostomum tenue* using its tail in similar fashion, but he likewise found no caudal adhesive glands.

C. conniae, C. argenti, C. dorotti, C. nolfi, and *C. aalbui,* have so many structural features in common that they seem to form a natural sub-group. The most striking similarities pertain to the stylet. In every case it is large, javelin-shaped, and does not have a basal bulb. The size of the shoulders varies a little, being heaviest in *C. argenti, C. dorotti,* and *C. nolfi.* In *C. conniae* and *C. aalbui* the wall is slightly thickened near the base. With the exception of these minor differences and the differences in length, the stylets of all five forms are very similar. Other similarities pertain to the general size and shape of body and tail, size of oral sucker and acetabulum and their dimentional ratio, the number and position of the penetration glands, the shape of the excretory bladder, and the spination.

There are significant differences, however, among them. *C. conniae* and *C. aalbui* are the only cercariae of the group that have a ring near the base of the stylet. They can be distinguished from each other by the abundance of refractile (i.e. oil-like) globules so apparent in *C. conniae* and by the larger size of *C. aalbui. C. argenti* has been found oftener than any of the five forms. It is characterized by the sheen of its cystogenous glands and their adhesion to the digestive system. *C. dorotti* is the only one in which the cystogenous glands completely hide the digestive system in emerged forms and make impossible the determination of the number and position of the penetration glands. *C. nolfi* has a large caudal receptacle with spines in the caudal sinuses. It is the only one to possess this trait. Cort (1915) created the sub-group Polyadena to include cercariae possessing the following characters:

1. Development in gastropods in elongate sac-shaped sporocysts.
2. Tail slender and less than the body length except when very much extended.
3. Acetabulum back of the middle of the body and smaller than the oral sucker.
4. Stylet about 0.30 mm in length, six times as long as broad, and with a thickening one-third of the distance from the point to the base.

5. Stylet glands, six or more on each side between the acetabulum and the pharynx.

6. Excretory bladder bicornuate.

7. Very short prepharynx and small pharynx present. Esophagus when developed short to of medium length. Intestinal ceca (when present) reaching to posterior end of body.

The five members of this group meet Cort's specifications except that the ceca of *C. conniae* and *C. argenti* do not reach to the posterior end of the body. All five resemble *C. isocotylea* in many respects, but differ from it both in the shape of the stylet and by having apparent cystogenous glands. Of previously described members of the Polyadena group they most resemble *C. acanthocoela* Miller, 1935, but differ from it in the exact size and shape of the stylet and in the occurrence together of spined caudal sinuses and short ceca.

It is proposed that the Conniae division of the Polyadena group be erected to include cercariae with javelin-shaped stylets without basal bulbs. It is believed by the author that life history studies will prove these cercariae to be closely related.

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LARVAL TREMATODES OF NORTHWEST IOWA. II. FOUR NEW STRIGEIDS[1]

F. G. Brooks

A number of new species of cercaria have been discovered by the author since he began his studies on the larval trematodes of the Iowa lakes region in 1940. In this region, which lies in the extreme northwestern part of the state, conditions are especially favorable for an abundant trematode fauna. There are many lakes, sloughs, and kettleholes of glacial origin. The region is on the flyways of migratory birds and is the nesting ground for many water and shore birds. The non-avian vertebrate life is likewise varied and plentiful.

The methods of handling and studying the cercariae did not differ greatly from those usually employed by workers in the field. Killing for measurement was done in hot 10% formalin and if a comparison was to be made with a related form previously described by another author, measurements were also taken by the same methods used by the other author if material was available at the time the comparison was being made.

In the present paper four new strigeids will be described and a taxonomic problem in connection with one of them will be discussed.

Cercaria stephensi n. sp.[2]
(Figs. 1, 2)

Specific diagnosis: Furcocercaria of Strigeid group. Four pairs of small penetration glands located lateral to acetabulum and in front of its mid-line. Prepharynx and pharynx about same length; esophagus forks well above acetabulum; ceca extend to region of bladder. No commissure in excretory system. Unpigmented "eyespots" lateral to fork of esophagus. Acetabulum muscular and protrusive. About thirty forward-pointing spines form three irregular rows in circumoral spineless area; oral cap covers nearly half of anterior organ; body thickly set with fairly heavy spines not in rows; spines in region of bladder and in front of acetabulum fewer but larger, resembling bristles; four rows of spines around opening of acetabulum; furcae bear spines in six longitudinal rows; tail-stem spineless but with flagellets; one pair of lateral body bristles. No caudal bodies; striated muscle fibers and cell nuclei show plainly in tail stem; no furcal finfolds.

Measurements: Measurements in millimeters of emerged living cercariae and of cercariae killed in hot 10% formalin:

	Living			Killed in 10% hot formalin		
	Extended	Contracted	Relaxed	Max.	Min.	Mean
Body length	0.300	0.114	0.184	0.210	0.140	0.177
Body width	0.042	0.090	0.054	0.044	0.076	0.056
Tail-stem length	0.240	0.182	0.196	0.216	0.124	0.180
Tail-stem width	0.040	0.040	0.040	0.040	0.036	0.038
Furcae length	0.288	....	0.220	0.238	....	0.192
Anterior organ length	0.048	0.032	0.038	0.045	0.036	0.038
Anterior organ width	0.032	0.032	0.032	0.025	0.020	0.024
Acetabulum diameter	....	....	0.036	0.038	0.028	0.032

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[1] From the Iowa Lakeside Laboratory and Cornell College.

The author is indebted to Professor T. C. Stephens of Morningside College, a member of the staff of Iowa Lakeside Laboratory, for the identification of the snails mentioned in this paper.

[2] Named in honor of Professor T. C. Stephens of Morningside College and the Lakeside Laboratory staff.

Precercarial stage: Cercariae produced in long, white sporocysts with straight sides. Attain length of more than six millimeters and are approximately 0.150 mm in diameter. There is a birthpore near the anterior end.

Host and Locality: C. stephensi was found in five of 94 snails of *Lymnaea stagnalis jugularis* collected at Rush Lake, 34 miles southeast of Lakeside Laboratory.

Activity.—This is a very active cercaria. In the water it vibrates its body rapidly and under a cover-slip it flexes itself laterally, practically forming a loop. This motion seems to aid it in losing its tail, which it disposes of rather quickly.

Cercaria leplei n. sp.[3]
(Figs. 3, 4)

Specific diagnosis: Strigeid cercaria with both body and furcae shorter than tail-stem. Two pairs of penetration glands which do not reach beyond midline of acetabulum. "Unpigmented eyespots" at sides of body just in front of penetration glands. Prepharynx short; ceca extend a short distance beyond acetabulum. Tail stem without caudal bodies or other prominent features; no furcal finfolds. Excretory system with commissure anterior to acetabulum. Two pairs of flame cells on each side empty into anterior and two pairs into posterior collecting tubes; one pair of flame cells in proximal part of tail. Oral cap composed of seven or eight rows of heavy spines which give way to lighter spines covering head organ; fine spines sparsely distributed over body; none observed on tail-stem, or furcae; three rows of heavy spines surround opening of acetabulum.

Measurements: Average measurements in millimeters taken from emerged cercariae killed in hot 10% formalin:

Body length	0.172	Oral sucker width	0.028
Body width	0.036	Acetabulum diameter	0.022
Tail-stem length	0.194	Pharynx length	0.012
Tail-stem width	0.033	Pharynx width	0.014
Furcal length	0.182	Middle acetabulum to posterior end	0.042
Oral sucker length	0.034		

Precercarial stage: Sporocysts long white tubes that superficially resemble threads. Longest measured was 7 mm. Parts of it empty measured 0.05 mm, while sections crowded with embryos were 0.08 mm in diameter. Birth pore occurs 0.07 mm from anterior end.

Host and Locality: Parasite of *Physella elliptica* Lea collected at Diamond Lake, ten miles north of Lakeside Laboratory.

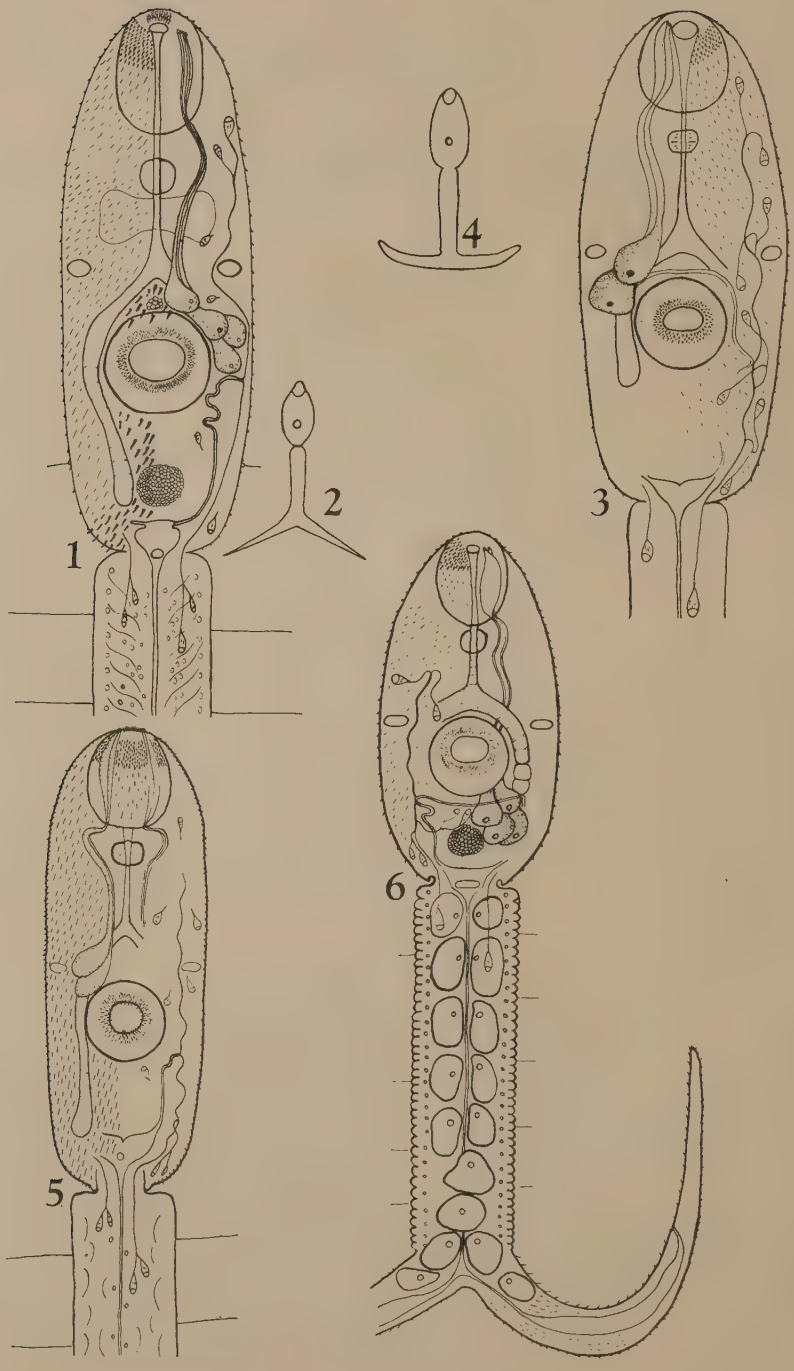
The relation of *C. leplei* to other species will be discussed together with that of *C. stonii* the description of which follows.

Cercaria stonii n. sp.
(Fig. 5)

Specific diagnosis: Strigeid cercaria with body and furcae slightly longer than tail-stem; oral sucker a little larger than acetabulum. Two pairs of small penetration glands anterio-lateral to acetabulum. Prepharynx short; esophagus and ceca easily seen; ceca extend to region of bladder. Unpigmented "eyespots" on each side near region of acetabulum. Two rows of forward-pointing spines of about ten spines each; oral hood of eight or nine rows; body and furcae covered with fairly long spines, not in rows; three or four rows of heavy spines surround opening of the acetabulum; flagellets on tail-stem. Excretory system without commissure; two pairs of flame cells in anterior third of stem. No caudal bodies or other distinguishing features in tail-stem or furcae.

Measurements: Bodies of living cercariae may attain length of 0.22 mm at extreme extension and may contract to 0.11 mm. Length of body, tail-stem, and furcae of moderately extended living cercariae measure 160 mm, 170 mm, and 210 mm. Average measurements in millimeters of emerged cercariae killed in hot 10% formalin:

[3] Named in honor of Mr. Charles Lepley of the Iowa Conservation Service.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Body length	0.196	Oral sucker length	0.037
Body width	0.058	Oral sucker width	0.031
Tail length	0.185	Acetabulum diameter	0.034
Tail width	0.032	Distance middle acetabulum to posterior end	0.066
Furcal length	0.198		

Precercarial stage: Sporocysts are long white tubes that separate readily from digestive gland of host. Have some power of movement, writhing when free in water. Large sporocysts measure 5 mm long by 0.18 mm in diameter; can be stretched to five times their normal length.

Host and Locality: C. stonii was found as parasite of *Lymnaea stagnalis jugularis,* collected in 1942 from Stony Lake, eleven miles west of Lakeside Laboratory.

Activity.—The activity of this cercaria when free in water is typical of strigeids. Under a cover slip it is not so active as most members of its group, showing a tendency to anchor with its acetabulum and stay in place.

The flame cell pattern could not be worked out. Fig. 5 shows eight found in the body. The flame cell near the anterior end and the one just posterior to the acetabulum are each probably one of a pair. If this is the case, the flame cell pattern would be $2[(2+2+2)+(2+2)+2]=24$.

C. leplei and *C. stonii* resemble each other in having two pairs of penetration glands anterio-lateral to the acetabulum. They differ in that *C. leplei* has a pre-acetabular excretory commissure while *C. stonii* is without an excretory commissure; *C. leplei* lacks forward pointing spines which *C. stonii* has; *C. leplei* has one pair of caudal flame cells while *C. stonii* has two pairs; and in that the ceca of *C. leplei* are much shorter than those of *C. stonii.*

Other strigeids without caudal bodies and having two anterio-lateral penetration glands and a preacetabular excretory commissure include *C. douglasi* Cort, 1917, *C. flabelliformis* Faust, 1917 (see Olivier and Cort, 1941), *C. sanjuanensis* H. M. Miller, 1927, *C. Strigeae tardae* Steenstrup (Mathias), 1922 (see Wesenberg-Lund, 1934, p. 117), *C. Cotylurus cornutus* Cercaria A of Szidat, 1923, and *C. fissicauda* LaVallette, 1855 (Brown, 1926). These differ from *C. leplei* by having two pairs of caudal flame cells and in various other features.

Other strigeids without caudal bodies having two anterio-lateral penetration glands and no excretory commissure include *C. marcianae* LaRue, 1917 (Cort and Brooks, 1928), *C. Neodiplostomum lucidum* LaRue and Bosma (West, 1935), *C. Pharyngostomum cordatum* Wallace, 1939, *C. Alaria mustelae* Bosma, 1934, *C. Alaria intermedia* Odlaug, 1940, *C. sincera* Olivier, 1941, *C. Fibricola texensis* Chandler, 1942, *C. indicae I* Sewell, 1922, and *C. tropicalis* Faust and Hoffman, 1934. Of these *C. Neodiplostomum lucidum, C. Pharyngostomum cordatum, C. sincera,* and *C. Fibricola texensis* differ from *C. stonii* by having only one pair of caudal flame cells; *C. marcianae* and *C. Alaria mustelae* lack forward-pointing spines and are much smaller; *C. Alaria intermedia* lacks unpigmented "eyespots," has shorter ceca and a differently shaped bladder.

Cercaria okobojensis n. sp.
(Fig. 6)

Specific diagnosis: Furcocercous cercaria of strigeid group. Prepharynx short; last two cells of ceca septate; ceca extend only short distance beyond acetabulum. Four pairs penetration

FIG. 1. *Cercaria stephensi* n. sp.
FIG. 2. *Cercaria stephensi* showing characteristic position of furcae.
FIG. 3. *Cercaria leplei* n. sp.
FIG. 4. *Cercaria leplei* showing characteristic position of furcae.
FIG. 5. *Cercaria stonii* n. sp.
FIG. 6. *Cercaria okobojensis* n. sp.

glands, all posterior to acetabulum, drained by two pairs of ducts which tend to form very large bulbs in anterior organ. Unpigmented "eyespots" near margin of body at level of anterior end of acetabulum. Oral cap covers less than half of anterior organ; no forward-pointing spines; body spines not in rows; furcal spines heavy, in eight longitudinal rows; four rows of prominent spines around acetabulum openings. Excretory system with post-acetabular commissure; six pairs of flame cells in body and one pair in tail-stem. Island of Cort short and broad. The two branches of excretory tubes which extend into furcae have tendency to become distended and bulbous. Fourteen distinct caudal bodies in tail-stem plus one large adjacent pair in furcae; largest caudal bodies measure about 30 by 22 microns, each with distinct nucleus. Flagellets about half as long as those seen in most strigeids, discernible in only a few specimens.

Measurements: Average measurements in millimeters of emerged cercariae taken in four different ways:

	Hot 10% formalin	Killed with heat	Mounted in balsam	Living
Body length	0.118	0.142	0.960	0.148
Body length extended	....	....	0.128	0.220
Body length contracted	....	....	0.080	0.080
Body width	0.047	0.560	0.036	0.056
Body width extended	....	....	0.028	0.310
Body width contracted	....	....	0.045	0.720
Tail-stem length	0.124	0.146	0.112	0.152
Tail-stem length extended	....	....	0.120	0.182
Tail-stem length contracted	....	....	0.105	0.094
Tail-stem width	0.036	....	0.030	0.038
Furcal length	0.137	0.170	0.130	0.172
Anterior organ length	0.029	0.036	0.026	0.036
Anterior organ width	0.023	0.025	0.020	0.026
Acetabulum diameter	0.023	0.026	0.021	0.028

Precercarial stage: Sporocysts are long, white tubes of even diameter. Mature cercaria-filled sporocysts measure 0.22 mm in transverse section and may be four or five millimeters long.

Hosts: Cercaria obtained from numerous snails of *Stagnicola umbrosa* Say, and *Physella elliptica* Lea.

C. okobojensis is by far the most common strigeid larval form in the Okoboji region. It was obtained from snails of most of the lakes and sloughs where collections were made in each of the three years in which work was done.

Nine previously described forms have four pairs of post-acetabular penetration glands. They are: *C. Apatemon gracilis* (Rudolphi, 1819) Szidat, 1931, *C. helvetica XXXI* Dubois, 1929 (see also Wesenberg-Lund, 1934), *C. hamburgensis* Komeya, 1938, *C. longiremus* Wesenberg-Lund, 1934, *C. gracillima* Faust, 1917, *C. burti* H. M. Miller, 1923 (see also Cort and Brooks, 1928), *C. pseudoburti* Rankin, 1939a and *C. caperata* Olivier, 1942.

C. longiremus has no commissure and lacks distinct caudal bodies. *C. helvetica XXXI* has an anterior rather than a posterior commissure, *C. gracillima* has 22 caudal bodies, and *C. caperata* has no commissure and has two pairs of flame cells in the tail-stem. The forms most similar to *C. okobojensis* are *C. burti, C. pseudoburti, C. apatemon gracilis,* and *C. hamburgensis.*

The writer recognizes that dimensions are unreliable especially in view of the fact that they are taken in so many different ways and that even when they are taken in the same way such factors as cover-slip pressure, temperature and strength of killing agent, and elapsed time since the larvae were killed affect the measurements. In order to compare dimensions as favorably as possible, ten or more cercariae were measured alive and after having been killed by heat, by 10% hot formalin and when mounted in balsam, thus duplicating the methods of each of the previous authors of closely related forms. The results tabulated under *C. okobojensis* and the compari-

sons which follow are made between measurements given by the previous authors and those of the new species taken the same way. Measurements of *C. okobojensis* differs from Miller's measurements for *C. burti* in that the body is much smaller. Since this form tends to contract until it is almost circular and at other times to distend itself greatly, three sets of measurements can be taken which will vary considerably. When the body of *C. okobojensis* is extended, its length is about the same as that given by Miller, but the tail-stem length is then considerably less than the body instead of being greater. The measurements for *C. okobojensis* differ from those given by Cort for *C. burti* principally in the length of the tail-stem. The ratio of Cort's measurements for the length of the body and tail-stem is 122: 165 while *C. okobojensis* was found to have the length of body and tail-stem nearly the same except when the body was in its contracted or extended condition. The width of the body of *C. burti* according to Cort's measurements indicates that it is a much larger form. Rankin's measurements of *C. pseudoburti* and Komiya's measurements of *C. hamburgensis* show a disproportionately larger tail-stem. Szidat's data for *C. Apatemon gracilis* indicate that it is a much larger form.

The present writer considers the difference in the position of the unpigmented "eyespots" as being significant. Both Cort and Rankin show these structures as being at the level of the fork of the esophagus. Szidat shows them midway between oral sucker and acetabulum, while Komiya shows complete absence of these structures. In *C. okobojensis* the "eyespots" are on a line with the front of the acetabulum or slightly posterior. They are also nearer the lateral margin of the worm than either Cort or Rankin show.

C. burti has four pairs of ducts leading from the penetration glands; *C. hamburgensis, C. pseudoburti* and *C. okobojensis* have two pairs. In the latter these are of fine caliber, but tend to become greatly distended in the anterior organ. Rankin shows and mentions ductal-distension, but the small nearly spherical bulbs that he shows were never seen in *C. okobojensis.*

The island of Cort in *C. okobojensis* is short and wide, while in *C. burti* and *C. pseudoburti* it is smaller and nearly circular and in *C. hamburgensis* and *C. Apatemon gracilis* it is absent altogether. The flame cell pattern in the five forms is the same.

C. okobojensis differs from *C. pseudoburti* in that the oral cap of spine covers less than half rather than three-fourths of the anterior organ. *C. pseudoburti* has two rows of irregularly spaced spines around the opening of the acetabulum. Cort says that the spines around the opening of the ventral sucker of *C. burti* are small and in two or three rows. *C. okobojensis* has four distinct and regular rows of fairly large spines.

Neither Miller, Cort, Rankin, Komiya, or Szidat shows well-marked individual cells in the oesophagus and ceca. These appear quite distinct in *C. okobojensis* and the last two cells of each cecum are septate.

Stunkard, Willey, and Rabinowitz, 1941, describe a cercaria having most features in common with *C. burti, C. pseudoburti,* the cercaria of *Apatemon gracilis,* and *C. okobojensis* and claim that the first three are the same, being the larval stage of *Apatemon gracilis.* Olivier, 1942, suggests that *C. helvetica XXXI* and *C. hamburgensis* may also be conspecific with the other three.

The form described by Stunkard, Willey, and Rabinowitz differs from *C. okobojensis* in that it has four instead of two ducts leading from the penetration glands,

a small, circular island of Cort, sixteen caudal bodies entirely in the tail-stem, and in that the body stem is distinctly shorter than the tail-stem (0.129 mm : 0.159 mm).

Giovannola, 1937, describes a form which he considers to be a variety of *C. burti* (*C. burti* var. *icnusae*). Apparently, the question is (1) whether the cercariae described by these various authors are a single cosmopolitan species whose small size is responsible for errors in description; (2) whether they are valid, but closely related species; or (3) whether they are distinct varieties of a single species. The present author believes that they represent at least several separate, but closely related species.

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LARVAL TREMATODES OF NORTHWEST IOWA. III. A NEW COLLARLESS ECHINOSTOME CERCARIA*

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The studies pursued by the author on the larval trematodes of the Lake Okoboji region in Iowa during the summers of 1940, 1941, 1942 have revealed a number of forms not previously described. In the present paper a new species of cercaria will be described which has the features of the echinostome group except that it lacks com-

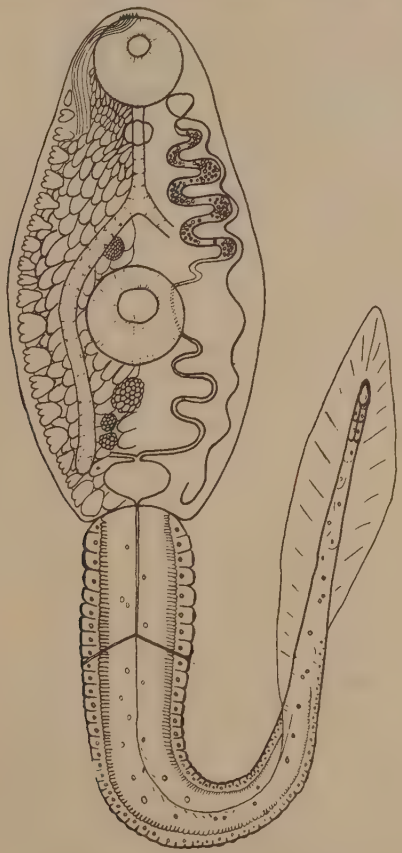

FIG. 1. *Cercaria ornatocauda* n. sp.

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pletely the usual collar of spines and is therefore referred to as being echinostomoid. The taxonomic relationships of this form are discussed at some length because of certain problems involved.

Cercaria ornatocauda n. sp.
(Fig. 1)

Specific diagnosis: Echinostomoid cercaria belonging to Reflexae group. Body very large, seldom with straight sides; acetabulum larger than oral sucker, both small; tail longer than body, with dorso-ventral finfold and very ornate. Cystogenous glands abundant containing granular but not rod-like material. Penetration glands not distinguished, but ducts seen leading to six pairs of nozzle-like openings in front of oral sucker. Prepharynx nearly as long as pharynx; esophagus forks well above acetabulum; ceca extend to region of bladder. Excretory bladder bulbous; siphons proceed from common opening at top of bladder and pass over acetabulum beyond which they form from four to seven undulations on each side of body before making reverse loops near oral sucker to become recurrent tubules; recurrent tubules ciliated throughout most of length; siphons anterior to acetabulum comparatively narrow and loosely filled with small refractive granules; excretory canal forks in upper sixth of tail. Genital primordia consists of three masses of cells located eccentrically between bladder and acetabulum and one mass in front of acetabulum. No spines on body or tail.

Measurements: This large, muscular larva changes size and proportions almost constantly when alive, but under a given killing procedure measurements are fairly constant. Living cercariae range from 0.340 × 0.306 mm contracted to 1.180 × 0.080 mm extended. Average measurements in millimeters of cercariae when living, when killed by heat, and when killed in 10% hot formalin:

	Living, moderately distended	Killed in 10% hot formalin	Killed with heat
Body length	0.680	0.560	0.672
Body width	0.270	0.220	0.275
Tail length	0.730	0.745	0.720
Tail width	0.085	0.072	0.085
Oral sucker length	0.062	0.064	0.068
Oral sucker width	0.066	0.058	0.068
Acetabulum diameter	0.085	0.088	0.088
Prepharynx	....	....	0.026
Pharynx length	....	....	0.029
Pharynx width	....	....	0.026
Center acetabulum to posterior end of body	....	....	0.285

Precercarial stage: Cercariae produced in typical rediae with gut extending to locomotor organs and containing golden or orange-colored materials. Walls of rediae contain yellow pigment. Typical size of rediae 0.900 × 0.340 mm.

Host and Locality: Found in numerous specimens of *Stagnicola umbrosa* Say, *Stagnicola palustris elodes* Say, and *Helisoma trivolvis* Say from various lakes and sloughs in the Okoboji region.

Activity.—When free in water, *C. ornatocauda* swims actively with a characteristic wobbling movement. It flexes its body to form a ball from which the tail extends for locomotion. Under a coverslip it loops its tail to form a lateral 8, and maintains with it a characteristic quivering motion. Emergence may occur any time during the day and only a few are found swimming in a bottle at a time.

Other echinostomoid cercariae of the reflexae group are: *C.* of *Psilostomum reflexae* Cort, 1914, also described by Feldman, 1941; *C.* of *Himasthla militaris* Rudolphi, described by Van Beneden, 1861; *C. helvetica XVII* Dubois, 1928; *C. penthesilia* Faust, 1921; *C. sudanensis I* Archibald and Marshall, 1931; and *Psilostomum ondatrae* Price, 1931, described by Beaver, 1939. Of these, *C. ornatocauda* most resembles *C.* of *Psilostomum reflexae*. A significant difference lies in the sizes of the two larvae and the dimensions of their parts. Feldman does not give

the size of the body, but Cort, 1915, says that a well-extended specimen has a length of 0.46 mm and a width at the acetabulum of 0.135 mm. Moderately extended specimens of *C. ornatocauda* average 0.680 × 0.270 mm. Both Feldman and Cort give 0.046 mm as the diameter of the oral sucker and Cort gives 0.060 mm and Feldman 0.071 mm as the diameter of the acetabulum; these structures in *C. ornatocauda* are 0.062 × 0.066 mm and 0.088 mm respectively. The tail length of *C. ornatocauda* is likewise much greater. Feldman mentions 42 paired flame cells plus a median unpaired one. The cystogenous glands of *C. ornatocauda* are so very dense that no flame cells could be seen. Neither Feldman nor Cort mentions anything comparable to the ornate "lacy" tail that is a conspicuous feature of *C. ornatocauda,* nor do their drawings indicate that the tail is highly ornate.

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EXPERIMENTAL STUDIES ON THE FISH HOSTS OF *POSTHODIPLOSTOMUM MINIMUM* (TREMATODA: STRIGEIDA)

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On the basis of experimental and morphological studies *Cercaria multicellulata* Miller, 1923, and *Neascus vancleavei* (Agersborg, 1926) were considered by Hunter (1937) and Ferguson (1937) to represent developmental stages of *Posthodiplostomum minimum* (MacCallum, 1921). Furcocercous cercariae described as *C. multicellulata* by Miller were obtained from *Physa gyrina* collected near Urbana, Illinois. Cercariae identified as belonging to the same species were found by Ferguson to infect *P. integra* near Urbana, Illinois, and by Hunter to infect *P. gyrina* in the lower Hudson watershed, New York State. Encysted metacercariae of the *Neascus* type collected in the United States and Canada from 23 species of fish, distributed over six families, were reported by Hunter and Hunter (1940) to represent the last larval stage of *P. minimum*. Similarly, the metacercariae of *P. minimum* were reported from 18 species of fish by Ferguson. The adult stage of this strigeid was described as *Diplostomum minimum* from the great blue heron (*Ardea herodias*) by MacCallum (1921). Adults collected from several species of heron in the United States and Canada, and those obtained from two species of herons and chicks experimentally fed encysted metacercariae from minnows and sunfishes, were considered by Ferguson to represent the species of *P. minimum*.

In recent studies metacercariae believed to be those of *P. minimum* from the pumpkinseed sunfish (*Lepomis gibbosus*), collected at Princeton, N. J., and blackhead minnows (*Pimephales promelas*), obtained from E. W. Surber, U. S. Bureau of Fisheries, Leetown, W. Va., were fed to two groups of trematode-free black-crowned night herons (*Nycticorax nycticorax*). Separate lots of laboratory-reared *Physa acuta* and *P. heterostropha* were exposed to miracidia hatching from the eggs of the adult strigeids developing in the herons from the metacercariae out of the minnow and sunfish hosts. Both species of snail became infected and released cercariae. Several dozen each of blackhead minnows and bluegill sunfish that had been held in the laboratory for several weeks were exposed to cercariae from *P. acuta* infected with the pumpkinseed line of strigeids. Similarly, several dozen each of blackheads and bluegills were exposed to cercariae from *P. acuta* infected with the blackhead line of strigeids. Bluegills exposed to cercariae of the sunfish line became infected and metacercariae were obtained after about 4 weeks, while blackheads similarly exposed were not infected. Bluegills exposed to cercariae of the blackhead line did not become infected, while blackhead minnows that were exposed became infected and fully developed metacercariae were obtained.

A small number of pumpkinseed sunfish, smallmouth bass, and largemouth bass after exposure to cercariae of the minnow line failed to become infected. However, after small numbers of the same three species were exposed to cercariae of the sun-

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fish line, only the largemouth bass failed to become infected. From the pumpkinseeds and smallmouth bass, encysted metacercariae were obtained.

Klak (1939) stated that at the U. S. Bureau of Fisheries Station, Leetown, W. Va., blackhead minnows, blunt-nosed minnows, spot-tailed minnows, and attractive minnows were infected with encysted metacercariae of the *Neascus* type. When smallmouth bass, largemouth bass, and golden shiners were taken from the ponds in which the above mentioned minnows were found to be infected, they never contained any of the larval strigeids. These observations in the field confirm the above experimental evidence which revealed that the minnow line of cercariae did not infect either largemouth or smallmouth bass.

The blackhead minnows, bluegill sunfish, and pumpkinseeds used in the experiments all contained a small number of mature cysts of the *Neascus vancleavei* type when they were exposed to cercariae of either the sunfish or minnow line released from *P. acuta.* However, within the limited range of these experiments no immunity reaction seemed to be expressed when minnows and bluegills were exposed to cercariae of the minnow and sunfish line respectively.

Encysted metacercariae from several species of minnows (CYPRINIDAE) and sunfishes and smallmouth bass (CENTRARCHIDAE) were studied in detail by Ferguson (1937) with particular reference to size, morphology, and host relationship. At the same time similar studies were made of adults recovered both from naturally infected herons and from herons and chicks fed metacercariae from fish representing the above two families. Live and fixed metacercariae from the minnows, while similar in morphological details to metacercariae from members of the family CENTRARCHIDAE, were uniformly smaller. Likewise adults developing in experimentally infected birds from the encysted larval worms obtained from minnows were uniformly smaller than those adults developing from the centrarchid line of metacercariae. It was found that the type of fixative and its temperature were factors which determined to a certain extent the sizes of the preserved metacercariae and adults. Usually there was an overlapping of the size ranges of metacercariae from representatives of the two families of fish. The same was true for adults developing from these two lines of metacercariae. In minnows the encysted helminths were found largely in the liver, on the mesenteries, or free in the body cavity, while in the sunfish and smallmouth bass they mostly occurred in the liver and kidneys and on the heart. Since the minnows were much smaller in size than the sunfish it was believed that the size of the host in relation to the number of parasites infecting it, and the position in the host's body in which the larval worms developed, must have been responsible for the ultimate size attained by the mature metacercariae.

The experiments reported here indicate that there is a definite specificity with regard to the fish hosts which the two lines of cercariae can infect. This is particularly interesting since the same species of snail and bird will serve as host for the two lines. It was not possible to study critically the morphology of the cercariae representing the minnow and sunfish lines of this strigeid material. Differences of taxonomic value may eventually be demonstrated to exist among these cercariae. If this is not done it will have to be assumed that physiological factors determine the fish hosts which are infected. Only when these questions are settled can it be decided whether two species of the genus *Posthodiplostomum* are being dealt with or whether physiological varieties are represented under a single species concept.

Since MacCallum described the adult stage of *P. minimum* from a heron dying in the New York Zoological Park, it is impossible to know the source of the metacercariae infecting this bird. Miller described *C. multicellulata* from *Physa gyrina* collected in Urbana, Illinois, and Hunter in New York State was able to obtain infections in sunfish exposed to cercariae from *P. gyrina* which he identified as belonging to the same species. Ferguson, at Urbana, Illinois, exposed blunt-nosed minnows to cercariae identified as *C. multicellulata,* and these larval worms developed into metacercariae. Since both the sunfish and minnow lines of trematode material were able to infect *P. acuta* and *P. heterostropha,* it is impossible to determine which line of cercariae Miller may have been dealing with. In view of the exposure experiments of Hunter with sunfish, and of Ferguson with minnows, both of whom believed they were working with *C. multicellulata,* apparently no significant difference was noted by these authors between their cercariae and the form described by Miller. However, further examination of these cercariae may reveal anatomical characteristics by which the lines may eventually be differentiated.

Neascus vancleavei was described by Agersborg from a species of minnow collected in Illinois and redescribed by Hughes (1928) from minnows collected in Illinois and sunfishes taken in Michigan. Hughes found that there was an overlapping of the size ranges of the metacercariae from the different fish hosts. Neither Hughes, Hunter, nor Ferguson detected any significant anatomical differences between metacercariae from the families Cyprinidae and Centrarchidae. If differences are eventually demonstrated between the metacercariae occurring in a bluegill sunfish and a blackhead minnow, those obtained from every species of fish in the two families mentioned will have to be carefully studied both morphologically and by infection experiments. If, as may be the case, there are at least two species or physiological varieties, it may be shown eventually that these forms are host specific as far as the family of the fish is concerned. Should two distinct species be proved to exist, only the metacercariae from minnows could be named *Neascus vancleavei.* In Hunter and Hunter, metacercariae considered to be those of *P. minimum* were reported from fish belonging to six families. The relation of all these metacercariae to one another would need to be investigated.

From the preceding discussion it becomes apparent that the life cycle relationships of MacCallum's adult worms and Miller's cercariae are unknown. The minnow origin of Agerborg's metacercariae is certain. In view of the uncertainties as to the specific status of the metacercariae now considered to be those of *P. minimum* it is evident that this confusion must be eliminated by morphological and experimental studies before the taxonomic status of the trematodes in question can be determined.

This confusion emphasizes once more that both morphological and life history experimental work must be done before it is possible to identify correctly stages in strigeid life cycles and evaluate specific concepts.

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A NEW MEDIUM FOR THE CULTIVATION OF *HISTOMONAS MELEAGRIDIS*

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Histomonas meleagridis, the protozoan parasite causing blackhead in turkeys, has been propagated in several different laboratory media since its discovery by E. E. Tyzzer in 1919.

Tyzzer (1) cultivated the organism in a medium composed of a buffered agar prepared with egg-albumin and covered with saline containing five per cent of sterile horse serum. Small quantities of rice starch and charcoal subsequently were added. This medium was reported to give good growth and to be somewhat less favorable to the growth of associated bacteria than the L.E.S. (Locke-egg-serum) medium of Boeck and Drbohlav (2). Bishop (3) cultivated the blackhead parasite in the inspissated horse serum slants of Dobell and Laidlaw (4). Cultures lasted five to seven days during a nine-month cultivation period. The serum slants were prepared by placing in culture tubes horse serum (sterilized by filtration) in suitable amounts and forming slants by heating to 80° C. The slants were covered with Ringer's solution containing four egg whites per liter. Previously Bishop had held cultures for an average period of three days at 37° C in a medium composed of inspissated whole-egg slants covered with a solution containing one part of horse serum in eight parts of Ringer's solution and with rice starch added before using. The short life of those cultures would appear to have resulted from the temperature employed rather than the type of medium, since the writer has held cultures of *Histomonas* on media of several kinds at 37° C but has never observed multiplication of the trophozoites at that temperature. Bishop reported a heavy overgrowth of bacteria as a result of using the liver medium of Cleveland and Sanders (5) and cultures died out after a few transplants. Bishop (3) also propagated the organism in a medium composed of one part of horse serum diluted 1:8 without a slope but with rice starch added before using (6). DeVolt and Davis (7) employed a L.E.S. medium with Locke's solution containing turkey serum and albumin or serum alone with subsequent additions of rice starch. Transfers were made every two or three days depending upon the relative numbers and kinds of associated bacteria in the cultures.

Experimental work designed to develop an improved medium for the cultivation of the blackhead parasite was undertaken by the writer in 1938. The necessity

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of adding sterile supplements to the media of the older types which were never completely sterilized in the autoclave proved troublesome and a handicap to the cultivation and further study of the organism. In addition, fragmentation of the egg slant by associated bacteria produced a colloid and shortened the life of the cultures. As observations indicated the protozoon did not obtain sustenance directly from the slant, that part of the medium appeared unessential. Since the propagation of the parasite in pure culture (in the absence of associated bacteria) is the ultimate goal, a medium that could be sterilized largely en masse was sought.

A new and improved medium for the cultivation of *Histomonas meleagridis* was developed by eliminating the slant and sterilizing serum in Locke's solution after adjusting the pH value of the solution. Rice starch is sterilized separately and added before using. The formula of the medium follows:

NaCl	9.0 gm
CaCl	0.2 gm
KCl	0.4 gm
$NaHCO_3$	0.2 gm
Glucose	2.0 gm
Fresh clear turkey serum	20.0 ml
Aqueous N/20 NaOH	20.0 ml
Distilled water	1000.0 ml

The medium is prepared by adding to Locke's solution two per cent of fresh turkey serum. Chicken serum is equally suitable and it is highly probable that the sera of other animals could be used as well in the preparation of this medium. Precipitation of the serum protein by heat in the autoclave is prevented by the addition of two per cent of aqueous N/20 NaOH as base. This adjusts the pH to approximately 9.0. Ten ml of the medium are placed in ordinary sized culture tubes (16 by 150 mm) and plugged with cotton. The medium is then autoclaved at 120° C for about 20 minutes. This heating period leaves the medium clear or produces not more than a barely perceptible turbidity which is well tolerated by the parasites. In selecting sera for the medium, care should be exercised to avoid the turbid sera commonly observed in fowls at the onset of the spring egg-production period. Such sera produce a stable colloid harmful to the parasites. The filtrate obtained after precipitating by heat clear serum in unadjusted Locke's solution has also supported cultures very well through several transplants.

Rice starch is sterilized in small tubes by dry heat at about 160° C for two hours. It is convenient to transfer a small quantity (one to two milligrams) of the sterile starch to culture tubes with a small scoop or spoon made from a piece of wire. A bowl is fashioned by flattening one end of the wire and turning up the edges. The bowl is then turned at right angles to the shaft which is fitted into the bottom of a small cork used to stopper the rice-starch tube. After heating the scoop in the flame of a bunsen burner the transfer is made after the mouth of both tubes have been flamed. A preliminary incubation period of 12 to 24 hours is sometimes used as a test of sterility of the completed medium.

If the parasite cultures are found to contain relatively large numbers of bacteria, interfering with the functions of the protozoon, it is well to eliminate glucose from the medium and reduce the concentration of serum to one per cent as both components are favorable to the overgrowth of bacteria. That certain bacteria exist with cultures of the blackhead parasite in some degree of symbiosis is shown by the

failure of *Histomonas meleagridis* to survive in cultures partially freed from bacteria by sedimentation. At the same time, trophozoites sedimented into the usual suspension of associated bacteria reproduced as before.

Cultures of the blackhead parasite have been propagated in the above medium for several periods of a year or more. Transfers ordinarily are made twice a week and viable organisms can be found in the medium after one or two weeks. The parasite has been isolated repeatedly from the cecal contents and on several occasions from the liver. In making liver cultures the surface of the organ is seared and a wire loop used to transfer three or four loopfuls of blood and hepatic tissue to each of a dozen or more culture tubes previously tested for sterility. Not more than one or two positive tubes have been found per dozen inoculated. The parasites have in every instance been found associated with mixed bacterial cultures.

The new medium offers the advantage of less frequent transfer, sterilization largely en masse, and easy preservation. A medium of this type lends itself more readily to investigations of the relationship between the protozoa and bacteria associated with them.

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OBSERVATIONS ON THE USE OF SEA WATER IN THE CONTROL OF *ANOPHELES ALBIMANUS* WIED.

HERBERT S. HURLBUT

In Puerto Rico, *Anopheles albimanus* breeds prolifically in certain coastal lagoons having varying concentrations of sea water. The degree of salinity is altered by tides, evaporation and rainfall. These factors operate to create favorable or unfavorable conditions for mosquito breeding according to the tolerance of the species. It has been recognized that *A. albimanus* will not tolerate high concentrations of sea water and in certain instances this mosquito has been controlled by the introduction of sea water into land locked coastal lagoons (Washburn, 1933, and Tullock, 1937). The investigation here recorded was made in order to obtain more precise information on the limiting concentration of sea water required for the control of this species and the means by which such a concentration can be maintained in certain natural breeding places.

It was found convenient to express the relative salinity in terms of per cent of sea water as determined by a hydrometer. Either a sodium chloride hydrometer or a urinometer may be calibrated from known dilutions of sea water. Eighty degrees Fahrenheit was taken as a convenient temperature and calibrations were made at five per cent intervals.

LABORATORY OBSERVATIONS

The per cent hatchability of eggs deposited by isolated females in known dilutions of sea water was finally chosen as the principal criterion for determining the limiting effect of sea water on the development of *A. albimanus*. The rearing of larvae introduced so many uncontrolled factors that data from this source proved to be less useful. All the ovipositing females were collected in nature within a radius of two miles. Specimens from each collection were divided so that examples were distributed to each of the test concentrations of sea water. The collections were made over a period of three months beginning in October and continuing through December.

Gravid or engorged females were isolated in shell vials approximately 25 mm in diameter and 100 mm long, containing about 25 mm of diluted sea water. The vials were lined in the lower two thirds with absorbent paper and stoppered with cotton. Dilutions of 25, 50, 75, and 80 per cent sea water were used. Deposited eggs were left in the vials for 7 days at approximately 80° F. They were then removed from the vial on the absorbent paper lining and the total number of eggs and

TABLE 1.—*Deposition and hatching of* A. albimanus *eggs in various dilutions of sea water*

Per cent sea water	No. females segregated	Per cent ovipositing	Per cent batches infertile	Av. per cent hatching per batch	Range per cent hatching	Av. No. eggs per batch	Range No. eggs per batch
Distilled water	120	33.3	20	65.5	0–100	113	16–211
25	103	31.1	3	33.1	0– 76	111	10–222
50	130	15.4	30	7.7	0– 32	97	13–194
75	74	20.3	73	6.6	0– 45	104	17–174
80	171	10.5	89	0.33	0– 5	86	27–184

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the number hatched determined by microscopic examination. The data are presented in Table 1.

The average per cent hatching per batch decreased with increasing concentrations of sea water. The average for distilled water was 65.5 per cent, for 25 per cent sea water 33.1, for 50 per cent sea water 7.7, for 75 per cent sea water 6.6 and for 80 per cent sea water 0.33 per cent. Twenty per cent of the batches deposited in distilled water were abnormal in that the chorion did not turn black after deposition. These eggs do not hatch. This phenomenon is observed quite frequently in eggs deposited in the laboratory. The relative number of such batches increased in the higher concentrations of sea water until in 80 per cent sea water only 11 per cent of the batches attained the black appearance of normal eggs and of these but a small percentage hatched (Table 1). The average number of eggs per batch did not appear to be affected significantly by the salt concentration but the number of mosquitoes actually ovipositing decreased in the higher concentrations of sea water. Under these experimental conditions one may expect about one third of the gravid females to oviposit on distilled water but only about ten per cent on 80 per cent sea water.

In two instances eggs from a single batch deposited on distilled water were divided after 5–15 hours and the fractions distributed on 66, 75, 80 and 100 per cent sea water. Under these circumstances the lethal concentration of sea water appeared to be between 66 and 80 per cent (Table 2).

TABLE 2.—*The effect of various concentrations of sea water on the hatching of eggs deposited by the same female. The eggs were deposited on distilled water and allowed to remain 5–15 hours before removal to diluted sea water*

Specimen		Distilled water	66 2/3% sea water	75% sea water	80% sea water	100% sea water
I	No. of eggs	21	21	X	21	20
	Per cent hatching	57	28	X	0	0
II	No. of eggs	48	X	44	46	X
	Per cent hatching	77	X	0	0	X

Table 3 gives the data obtained from laboratory rearing of *A. albimanus* in diluted sea water and in tap water. Dried yeast was supplied as food. The eggs in each case were deposited at one time by a segregated female on the concentration of

TABLE 3.—*Rearing of* A. albimanus *in diluted sea water*

Per cent sea water	No. of eggs	Per cent hatching	Per cent reaching adult stage	No. of days to complete cycle
0	61	67	33.0	14
33	123	80	21.0	12
33	163	45	6.0	12
75	131	15	2.0	16
75	115	45	0.9	17

sea water indicated. The salt concentration was maintained approximately the same during the larval and pupal stages by adding distilled water to compensate for evaporation. The uncontrolled factors introduced in rearing the larvae make the data of limited value. However, it does indicate that this species is capable of passing through all its aquatic stages in concentrations of sea water as high as 75 per cent.

The survival of 1st instar larvae collected from a brackish water breeding place was also studied when they were subjected to higher concentrations of sea water. Sea water was added to the water taken from the natural breeding place until estimated concentrations of 50, 75, 80 and 90 per cent were reached as determined by a hydrometer. The water from the natural breeding place was estimated to be the equivalent of about 25 per cent sea water. Yeast was utilized as food. The data are presented in Table 4. Twelve per cent of those in 80 per cent sea water attained the adult stage but none of those in 90 per cent sea water survived beyond the second instar.

TABLE 4.—*Survival of* A. albimanus *larvae collected in 25% sea water when subjected to higher concentrations*

Per cent sea water	No. of 1st instar larvae	Per cent reaching pupal stage	Per cent reaching adult stage
25	17	82	65
50	17	65	6
75	17	35	18
80	16	19	12
90	15	0	0

FIELD OBSERVATIONS

An opportunity for observing the effect of gradually increasing salinity on *A. albimanus* production in a natural breeding place was afforded by the introduction of sea water into a coastal lagoon where this species was breeding in abundance. Table 5 gives the rate of increase in salinity as determined by almost daily hydrometer readings. Larval densities were determined by counting the number of larvae per 40 dips in a selected area of approximately 100 square feet. The salinity samples were taken from the same location. The daily collections of larvae classified according to instar are recorded in Table 5. All larvae were returned to the

TABLE 5.—*The effect of increasing sea water concentration on the production of* A. albimanus *in a natural breeding place*

Date	Per cent sea water	Larval instars					*A. albimanus* identified	Remarks
		1	2	3	4	Pupae		
Oct. 10	15	..	..	..	..	..	..	.....
11	20	..	..	..	..	..	..	.....
12	25	50	3	5	10	0	68	.....
13	30	..	..	..	..	..	..	.....
14	35	..	..	..	..	..	..	Oil applied
16	50	..	..	..	..	..	..	.....
19	65	18	0	0	0	0	..	Some newly hatched
20	65	4	13	0	0	0	..	
21	75	0	11	2	0	0	..	.....
22	65	1	7	16	1	0	..	.....
23	70	0	0	2	1	0	1	.....
24	75	0	0	1	3	1	2	.....
26	75	0	0	0	2	0	2	.....
27	85	0	0	1	4	0	3	.....
29	80	0	0	0	2	2	4	.....
30	90	0	0	0	1	0	2	1 pupal skin
31	85	0	0	0	0	1	1	.....
Nov. 2	90	0	0	0	1	1	2	.....
4	85	0	0	0	0	0	..	1 *A. grabhami*
5	5	..	..	..	..	..	..	4.5 in. rain
6	15	0	0	0	0	0	..	.....
8	20	0	1	0	0	0	1	.....
10	40	1	1	0	0	0	2	.....
12	65	0	0	0	0	0	..	.....
14	85	0	0	0	0	0	..	.....
16	90	0	0	0	0	0	..	.....
18	85	0	0	0	0	0	..	.....

breeding place except the collections of October 12th and 19th. Identification indicated that the larvae were almost 100 per cent *A. albimanus*. It will be observed from Table 5 that practically no 1st instar larvae were encountered after October 20. This would indicate either that oviposition ceased about October 15 when the relative salinity was about 50 per cent, or eggs deposited after that time failed to hatch. However, the larvae already present continued to develop and an observable number reached maturity even though the relative salinity had by that time exceeded 80 per cent. These observations agree reasonably well with those made in the laboratory. Continued dipping throughout the marginal area of the lagoon indicated that the production of *A. albimanus* had been virtually eliminated.

Prior to the establishment of a sea connection, collections made over a period of six weeks indicated the presence of *A. albimanus* in abundance with the relative salinity remaining between 15 and 25 per cent sea water. Concurrent samplings in an adjacent lagoon separated from the first by a roadway fill and communicating with the sea by a small channel were entirely negative. The salinity here varied between 70 and 80 per cent.

It appears from the foregoing observations that active breeding of *A. albimanus* ceases in water having a salinity much exceeding that of 50 per cent sea water. The continued presence of larvae probably indicates that the salinity has recently been much lower or the larvae have been washed in from some fresh water habitat.

MAINTAINING THE DESIRED SALINITY IN NATURAL BREEDING PLACES

In practice, it is desirable to maintain the salinity high enough to allow an adequate margin of safety above the point where active breeding ceases. Seventy-five per cent sea water is believed to furnish this margin. Tidal action in the lagoon was found to be the most potent factor in distributing the salt to remote shallow areas where mosquito breeding is most likely to occur. Daily tidal fluctuations of 5 or 6 inches were obtained in the lagoon under observation. This resulted in a rapid increase in salinity until it stabilized between 80 and 90 per cent (Table 5). Four and one half inches of rain on November 5th brought it as low as 5 per cent but within a week it had returned to the former stabilization level.

The lagoon under observation had an area of approximately 400 acres and an average depth of about 1.5 feet. It was divided along its long axis into two nearly equal parts by roadway fills. An opening to the sea 55 feet wide and another 30 feet wide through the roadway fills proved to be adequate to produce the required tidal action and thereby maintain the necessary salinity. The average tidal range in this locality is 0.8 foot. The distance from the sea outlet to the most remote part of the lagoon was about two thirds of a mile.

OTHER ASPECTS OF TIDAL ACTION AND INCREASED SALINITY

No doubt, the mechanical action of the daily water level fluctuation exerted beneficial effects independent of salinity by stranding flotage and exposing the larvae to their natural enemies through disturbance of the surface cover. Top feeding minnows were numerous. The beneficial influence of water level fluctuation in the control of *A. quadrimaculatus* in impounded water has been reported by Hinman (1938).

The increased salinity and tidal action produced marked changes in the flora. No detailed study was made of this but it was observed that a very troublesome plant, widgeon grass (probably *Ruppia maritima*) stopped spreading and was largely eliminated. It grows submerged in shallow brackish water forming dense mats at the surface. This makes a particularly favorable habitat for *A. albimanus*.

SUMMARY

1. *A. albimanus* breeds abundantly in certain brackish coastal lagoons in Puerto Rico.

2. Breeding of this species can be eliminated by increasing the relative salinity to about 75 per cent sea water.

3. This degree of salinity can best be maintained in marginal areas of lagoons by tidal action produced by an adequate sea connection.

4. A technique is described by which the tolerance of a mosquito for different salt concentrations can be tested by observing the effect on the hatching of the ova.

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RESEARCH NOTES

NOTES ON SOME PARASITES OF THE MINK IN SOUTHERN MICHIGAN

As a part of a more complete study dealing with the mink (*Mustela vison mink*) the writer autopsied 158 mink carcasses which were obtained from southern Michigan fur-buyers during the winters of 1940 and 1941. In addition to measurements taken on the carcasses, an effort was made to determine the incidence of some of the more important macroscopic parasites.

From a wildlife management standpoint it is important to know the incidence of parasites in various animals in order to evaluate the rôle which they play in regulating animal populations. There is still relatively little known concerning some of the parasites infecting our wild animals and the effects which they produce. Until recent years the approach has been largely taxonomic and morphologic, but if cyclic phenomena and trends of animal populations are to be correctly interpreted. the proportion of the population infected and the severity of the infections from year to year must be known. Although it is not to be expected that parasites infecting animals in the wild state can be controlled to any great extent, a knowledge of their distribution, incidence, life cycles, and modes of transmission may serve to prevent them from becoming a menace to ranch-bred animals.

The mink spends a considerable portion of its time in or near water and hence many aquatic animals eaten by mink, such as crustaceans, frogs, and fish, act as intermediate hosts for the large assortment of flukes and roundworms which habitually infect it. In common with the muskrat, the mink is often referred to as a virtual "museum of parasites."

Thirty-two carcasses were autopsied in 1940 and 126 in 1941, and the incidence of the most frequently encountered parasites is presented in Table 1. No significant differences in the two years were shown when the chi square test was used.

TABLE 1.—*The incidence of some helminthic parasites found in mink taken during the trapping seasons of 1940 and 1941*

Species	Year	Number infected	Incidence (%)	Average incidence two years (%)
Skrjabingylus nasicola	1940	32	100	94
	1941	116	92	
Filaroides bronchialis	1940	3	9	19
	1941	27	21	
Paragonimus kellicotti	1940	2	6	9
	1941	13	10	
Physaloptera sp.	1940	0	0	4
	1941	6	5	
Dioctophyme renale	1940	1	3	3
	1941	3	2	

The sinus worm, *Skrjabingylus nasicola,* appears to be the most common nematode parasite of the mink. The worms which occur in the frontal sinuses of the animal are frequently overlooked. In heavy infections the frontal sinuses were inflated and discolored by the mass of worms within. A similar condition commonly noted in skunks is caused by the related species, *S. chitwoodorum.* In one mink 45 of these worms were recovered from the two sinuses although the usual number found was about a dozen individuals. In a few instances the worms had apparently bored out through the wall of the sinus into the overlying muscle.

Lungworms, *Filaroides bronchialis,* are perhaps next in importance to the sinus worm. They occur as small, compact knots of closely intertwined worms which are usually situated along the trachea and bronchi and on the surface of the pulmonary vein. A few of these knots of worms were occasionally noted on the surface of the lung, and as many as six were found in the lungs of one mink.

The lungfluke, *Paragonimus kellicotti,* which is an occasional parasite in the lungs of cats, dogs, and pigs, finds its natural definitive host in the mink. Various species of crayfish belonging to the genus *Cambarus* constitute the second intermediate host while the snail, *Pomatiopsis lapidaria,* is the first (Ameel, 1934, Am. J. Hyg. **19**: 279–317). An incidence of 8.09 per cent is reported by Wallace (1931, Am. Fur Breeder, **3**: 24–25) from 84 carcasses received from fur farms in Minnesota. This compares closely with the 9 per cent incidence of infection which the author found in wild mink. Four cysts were found in the lungs of one mink with as many as six flukes to a cyst, but usually only a single cyst was found.

Larval and adult *Physaloptera* sp. occurred in the stomachs of six minks, one of them containing ten. In an animal heavily infected with this parasite the wall of the stomach was very much thickened.

The giant kidney worm, *Dioctophyme renale*, was found in only four of the 158 mink which were autopsied. Though not occurring as frequently as other parasites this worm is probably more destructive in the wild because of the total damage which it inflicts on the infected organ. In one of the parasitized animals a compensatory hypertrophy of the uninfected kidney was noticeable. A large calculus was present in the kidney of another.

Several other parasites were recorded from the minks which were examined, but they were probably of little importance. Coccidia, *Isospora bigemina*, and the eggs of *Capillaria* sp. were frequently noted in fecal samples together with larvae and adults. An adult female capillarid was recovered from the stomach of one mink.

Acknowledgment is made to Mr. J. T. Lucker of the Zoological Division, Bureau of Animal Industry for aid in identifying some of the parasites and for confirming the author's identifications, and to Mr. B. T. Ostenson of the Zoology Department, Michigan State College for general supervision.—JOHN A. SEALANDER.

TWO FLATWORMS FROM THE OYSTER-DRILLING SNAIL, *THAIS FLORIDANA HAYSAE* CLENCH

During the course of investigations on the salinity tolerance of the Gulf Coast oyster-drilling snail, *Thais floridana*, made during the spring of 1942, the author had occasion to observe two associated flatworms. Polyclad turbellarians were obtained frequently from Barataria Bay, La. snails, *T. floridana haysae* Clench, and occasionally from those of Santa Rosa Sound, Fla., *T. floridana floridana* Conrad. They emerged from the mantle cavities of their hosts after the snails had been immersed in water of low salinity, 10 p.p.1000 and below, for a few days. Some Barataria Bay specimens have been identified by Dr. Libbie Hyman as *Hoploplana inquilina* Wheeler, 1894. In a communication of May 28, 1942, Dr. Hyman stated that two of the specimens exceed the maximum size recorded for the same species from Woods Hole, Mass. (Hyman, L. H., 1940, Proc. U. S. Nat. Mus. **89**: 449–495). Pearse and Wharton (1938, Ecol. Monographs **8**: 605–655) have reported *"H. thaisana,"* which may or may not be the same as *H. inquilina*, from *T. floridana floridana* Conrad obtained in Apalachicola Bay, Fla. Stauber (1941, J. Parasitol. **27**: 541–542) discovered *Hoploplana inquilina thaisana* Pearse, 1938, in two species of snails associated with the oyster beds of Delaware Bay. He, too, observed that the worm abandoned its host at low salinities. Since the Delaware Bay and Florida specimens are both smaller than those of Woods Hole it appears that the largest of all are to be obtained in the Barataria Bay region. It is interesting to note that *Thais* of this region is also of extremely large size. Barataria Bay snails were also occasionally infected with trematode cercariae, which emerged when the snails were immersed in water of relatively high salinity, 25 p.p.1000 down to 14 p.p.1000. Specimens were submitted to Dr. H. W. Stunkard and identified by him as *Parorchis acanthus* (Stunkard, H. W. and Cable, R. M., 1932, Biol. Bull. **62**: 328).

In so far as the author is aware neither *Hoploplana inquilina* nor *Parorchis acanthus* has previously been reported from the Barataria Bay region, west of the Mississippi River, nor from the host with which they are there associated. It is of some interest that they appear to have been enabled to cross the natural barrier of fresh water with the aid of transplantation methods used in cultivating Louisiana oysters (McConnell, J. N. and Kavanagh, L. D., 1941, Bull. No. 1, State of Louisiana, Department of Conservation).—VICTOR SCHECHTER, *City College of New York, and the United States Fish & Wildlife Service Laboratory, Pensacola, Florida.*

THE SELECTIVE ACTION OF SULFAGUANIDINE ON AVIAN COCCIDIA

Previous reports have indicated that sulfanilamide and sulfapyridine were inhibitory for those species of chicken coccidia which completed their entire development in the small intestine (Levine, 1939, Cornell Vet. **29**: 309–320; 1940, J. Parasitol. **26**: 233–235). In these studies neither *Eimeria tenella* nor *E. necatrix*, the oöcysts of which are formed in the ceca, were adversely affected. Investigation of other sulfonamide compounds led to the discovery that sulfaguanidine, when fed to chickens in concentrations of 0.5% of the ration, apparently suppressed completely all the species with the exception of those mentioned. The severity of infections with those two species was markedly reduced by increasing the concentration of the drug to 1.0% and 1.5% respectively (Levine, 1941, Cornell Vet. **31**: 107–112; 1942, Cornell Vet. **32**: 430–439).

Since the quantitative methods used in these studies did not permit recognition of infections when fewer than 40 oöcysts per gram of feces were present, the following critical experiments

were done to determine whether infections could be completely prevented by the feeding of sulfaguanidine. Two chickens, reared coccidia-free, were dosed with a mixture of pure "cultures" of sporulated oöcysts of all the species affecting chickens; *E. acervulina, E. praecox, E. mitis, E. hagani, E. brunetti, E. maxima, E. tenella* and *E. necatrix*. One of the birds had been fed mash containing sulfaguanidine in the proportion of 0.5% by weight for 48 hours previous to the infective dosing. This bird was kept on the medicated feed for the duration of the experiment. The second bird received non-treated mash and served as a control. Daily fecal examinations (sugar flotation) were made.

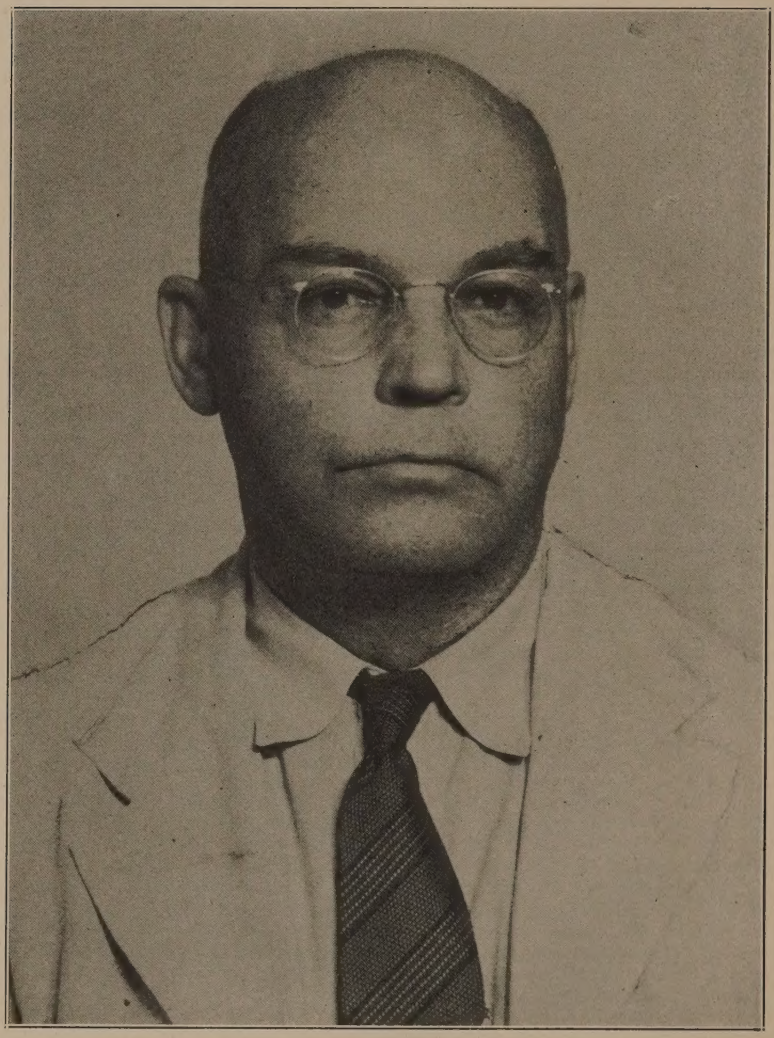
The control bird became infected with all of the species as evidenced by the appearance of their oöcysts in the feces, those of *E. praecox* and *E. acervulina* on the fourth day and others later. Post-mortem examination on the eighth day revealed the presence of typical lesions of *E. tenella* and *E. necatrix*. Oöcysts were not found in the feces of the treated bird until the seventh day at which time oöcysts similar to those of *E. tenella* and *E. necatrix* were observed. A bloody fecal discharge had been noted from this bird five days after the feeding of oöcysts indicating that infection with *E. tenella* had taken place. To establish the identity of these oöcysts, about ten grams of feces from the treated bird were collected on the eighth day and incubated for three days in shallow layers of 1½% potassium dichromate solution at 30° C to effect sporulation. If species other than *E. tenella* and *E. necatrix* were present (but missed on fecal examination), their oöcysts would surely be in this fecal sample since their life cycles are shorter. The incubated feces were fed to two coccidia-free chickens and again daily fecal examinations were done. It is generally agreed that this is the most delicate method of detecting the presence of oöcysts which may have been missed in routine fecal examinations. When no oöcysts were found up to and including the sixth day after dosing, one of the birds was destroyed for examination. Gross lesions typical of infections with *E. tenella* and *E. necatrix* were observed. Significantly enough, scrapings of mucosa from the small intestine when examined microscopically, were not only free from oöcysts but also from the intermediate stages of all of the other species. Examinations of feces from the remaining bird showed the presence of oöcysts on the seventh day and once more infections with only *E. tenella* and *E. necatrix* were confirmed. This experiment was twice repeated with identical results.

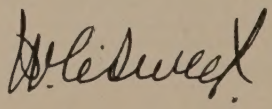
It is apparent that when sporulated oöcysts of *E. acervulina, E. praecox, E. mitis, E. hagani, E. brunetti,* and *E. maxima* are ingested by chickens being fed sulfaguanidine in concentrations of 0.5% of the ration, infection is completely prevented. The absence of intermediate stages from the intestinal mucosa indicates that the destruction of the inocula may have occurred even before invasion of the epithelium was effected. The fact that *E. tenella* and *E. necatrix* survive, makes this method valuable for the isolation of these species.—P. P. Levine, *New York State Veterinary College, Ithaca, N. Y.*

A NEW LOCALITY FOR *TRYPANOSOMA VESPERTILIONIS* (=*T. CRUZI* ?) IN BATS IN THE UNITED STATES

Dias (1937, Tr. Roy. Soc. Trop. Med. & Hyg. **31**: 260) reported a trypanosome morphologically similar to *Trypanosoma* (*Schizotrypanum*) *cruzi* from the Pacific pallid bat, *Antrozous pallidus pacificus*, from Pinole, Contra Costa county, California. The writer believes that the extensive experience of Dr. Dias with bat trypanosomes justifies his identification. Since no one has yet proven conclusively that this organism is *cruzi* by demonstration of its host relationships, the writer believes it is more accurate to call it *Trypanosoma vespertilionis*.

On July 13, 1941, one of my former students, Robert O. Ingman, made two blood smears from an adult female *Antrozous pallidus pacificus* from seven miles west of Plymouth, Amador county, California. Examination of both films reveals two trypanosomes on one slide identical with *Trypanosoma vespertilionis* as illustrated by Wood (1941, Am. J. Hyg. **34**: 4). This observation emphasizes the importance of investigating thoroughly the great central valley of California, especially the Sierra Nevada foothill areas, for demonstration of the causative agent of Chagas' disease in mammals and blood-sucking arthropods. Wood (1942, Los Angeles City Coll. Prog. Bull. **1**: 31–34) has already reported the existence of *Trypanosoma cruzi* in *Triatoma protracta* from human habitations at Trimmer Springs, Fresno county, California.—Sherwin F. Wood, *Department of Life Sciences, Los Angeles City College, Los Angeles, California.*





IN MEMORIAM

Winfield Carey Sweet (1891–1942)

On May 20, 1942, Dr. Winfield Carey Sweet died suddenly of a heart attack in Cochabamba, Bolivia. He was born in La Crosse, Wisconsin, on December 21, 1891. Until he was sixteen he lived in China, where his parents were missionaries. His collegiate training was in Colgate University from which he graduated in 1913, and he received his medical degree from Rush Medical College in 1917. Later he studied at the Johns Hopkins School of Hygiene and Public Health, receiving the M.P.H. degree in 1927 and the Dr.P.H. in 1931.

Dr. Sweet was a medical officer in the U. S. Army from July, 1917, to June, 1919, spending nine months in Italy. After the war he served for nine months with the American Red Cross in Siberia and then went to the Christian Hospital, Shoahsing, Chekiang Province, China, where he took the place of a staff member on leave.

Dr. Sweet became a member of the field staff of the International Health Division of the Rockefeller Foundation on November 10, 1921. His first assignment was to Australia where he assisted Dr. W. A. Sawyer in developing a hookworm campaign, which later broadened out to include surveys on malaria and filariasis. This gave him the interest in parasitology which continued throughout the rest of his career. His next post was in Ceylon, where he directed the hookworm and rural sanitation program until the end of 1925. Then he returned for a year and a half study leave at the Johns Hopkins School of Hygiene and Public Health. During this period, he spent four months in Panama on hookworm investigations with Dr. W. W. Cort. In June, 1927, he went to India where he was stationed for the next twelve and one-half years, except for another year of study leave in Baltimore. Until 1938, his headquarters were in Bangalore in the State of Mysore. His work in India began with a hookworm and malarial survey. Later he served as a consultant to the government on public health. While in Bangalore he also maintained supervision over the health programs in Madras and Travancore. During his last eighteen months in India, he was stationed first in Delhi and then in Calcutta.

In February, 1940, Dr. Sweet was transferred to China to take charge of a program of investigations of malaria on the Burma Road. In the summer of 1941 he returned to the United States on leave expecting to go back to China, but was prevented by the outbreak of the war with Japan. Instead, he was given an assignment in Bolivia and left for South America on April 2, 1942. After a short stay in Buenos Aires he started his work of organizing the new hookworm and malaria programs for the Bolivian Health Department on May 6, only two weeks before his death.

Dr. Sweet was fundamentally interested in research and he found time in spite of extensive administrative duties not only to carry on investigations but to publish his results. Of his forty-four papers, all but three are in parasitology. They are chiefly concerned with problems of malaria and hookworm disease in the countries where he worked. In his researches on malaria he studied not only the distribution, epidemiology and control of the disease, but also the mosquito vectors. He also made important contributions on the epidemiology and control of hookworm disease

in Australia and India, and the researches which he and Dr. V. N. Moorthy made on the guinea worm are fundamental contributions on the biology and epidemiology of this parasite. All his work showed the happy combination of a very practical public health viewpoint with a sound biological approach.

Dr. Sweet well exemplified the spirit of the organization that he represented, combining the missionary with the scientific spirit. He was modest and unassuming, while forceful and effective in his work. He was truly a citizen of the world and served as an ambassador of good will by bringing the best of American public health progress to the peoples with whom he worked. His untimely death at the height of his career is a great loss since men of his type were never more needed than at the present time. He will be missed by his many friends all over the world.—W. W. Cort, *Department of Parasitology, Johns Hopkins School of Hygiene and Public Health.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Notice of Postponement of Annual Meeting of the Society

On July 15, 1943, Mr. Joseph B. Eastman, Director of the Office of Defense Transportation of the Office of Emergency Management, Washington, D. C., issued a statement with respect to the effect that conventions and group meetings have upon the nation's transportation facilities and urged that whenever possible such gatherings be postponed or cancelled. His statement follows, in part:

> ". . . Conventions, even small conventions, produce concentrations of passenger traffic which severely impair the ability of the railroads and bus lines to provide adequate passenger service for military and essential war business travel. The passenger transportation problem is now so serious that I feel obliged to renew and reiterate, in the strongest terms, my request for cancellation of all such gatherings. . . .
>
> "Each organization must make its own independent decision. The Office of Defense Transportation cannot pass upon the essentiality of any proposed meeting. It can only emphasize the serious burdens which convention and group-meeting travel imposes on transportation and request voluntary action by those who have it in their power to eliminate such travel. . . ."

In accordance with Mr. Eastman's request, the Council of the American Society of Parasitologists has voted to postpone the next annual meeting of the Society until travel restrictions are lifted.

James T. Culbertson,
Secretary.

August 18, 1943.